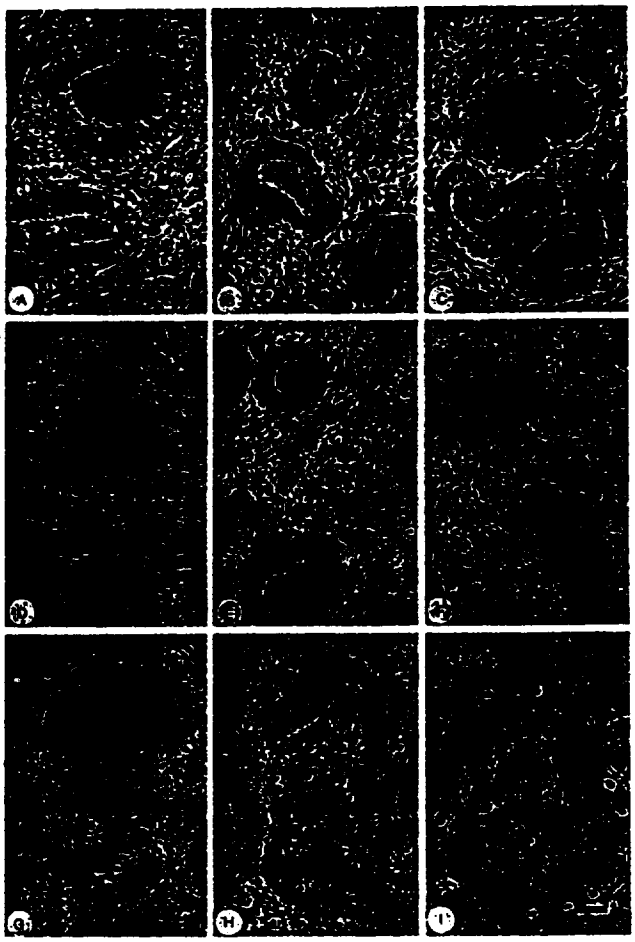

Figure 4 Photomicrographs showing immunolocalization of leukemia inhibitory factor, 15-hydroxyprostaglandin dehydrogenase, and Ki 67 on LH+6 of control cycle (A, D, and G) and after treatment with onapristone (B, E, and H) and mifepristone (C, F, and I). Scale bar, 50 μm. (A), Secretory endometrium from LH+6 of control cycle displayed intense leukemia inhibitory factor immunoreactivity (*brown*) in cytoplasm of glands and moderate immunostaining in stroma. Occasional discrete stromal cells (*arrow*) observed to exhibit leukemia inhibitory factor immunoreactivity. Endometrium taken 4 days after onapristone (LH+6) (B) and 4 days after mifepristone (LH+6) (C) displayed faint or absent leukemia inhibitory factor immunoreactivity in cytoplasm of glands and moderate immunostaining in stroma. (D), Endometrium on LH+6 of control cycle displayed positive 15-hydroxyprostaglandin dehdrogenase immunoreactivity (*brown staining*) in cytoplasm of glands. Endometrium 4 days after onapristone (LH+6) (E) and 4 days after mifepristone (F) shown with reduced 15-hydroxyprostaglandin dehydrogenase immunostaining. (G), Secretory endometrium from LH+6 of control cycle shown with very occasional Ki 67 immunostaining (*brown*) in glands and stroma. Endometrium taken 4 days after onapristone (LH+6) (H) and 4 days after mifepristone (LH+6) (I) displayed considerable Ki 67 immunostaining (*brown*) in nuclei of both glands and stroma.

LH+12 after administration of mifepristone ($P < 0.005$) (Fig. 5).

DISCUSSION

The human blastocyst is believed to enter the uterine cavity approximately 4 days after ovulation and to begin implantation at around the 6th day (17). The histologic appearance and immunohistochemistry of the endometrial biopsies taken at these stages of the cycle (LH+4 and LH+6) after treatment with the antiprogestins would suggest that uterine receptivity had been affected adversely. Histologically, the endometrium was retarded and secretory changes were either absent or poorly developed. These findings are consistent with previous studies using mifepristone (3, 4) and onapristone (5).

Our finding of leukemia inhibitory factor immunostaining in cytoplasm of both glands and stroma of secretory endometrium from control cycles, together with increased immunoreactivity in glandular epithelium around the expected time of implantation (LH+6), confirms a recent report regarding the immunolocalization of this cytokine in endometrium (9) and is in contrast to a previous report that it was confined to epithelium (8). It has been demonstrated previously that certain lymphoid cells within the decidua express leukemia inhibitory factor mRNA (18). In view of this, it is possible that the stromal cells in control-cycle biopsies that were observed to exhibit discrete leukemia inhibitory factor immunoreactivity are also hemopoietic cells. Although leukemia inhibitory factor has been shown to be critical for implantation in mice (19), its precise biologic role in the human is still uncertain. However, the temporal expression of leukemia inhibitory factor in endometrium and the coexpression of its receptor in the embryo would suggest that leukemia inhibitory factor may also play a role in the implantation process (8). Indeed, recent studies also have demonstrated that leukemia inhibi-

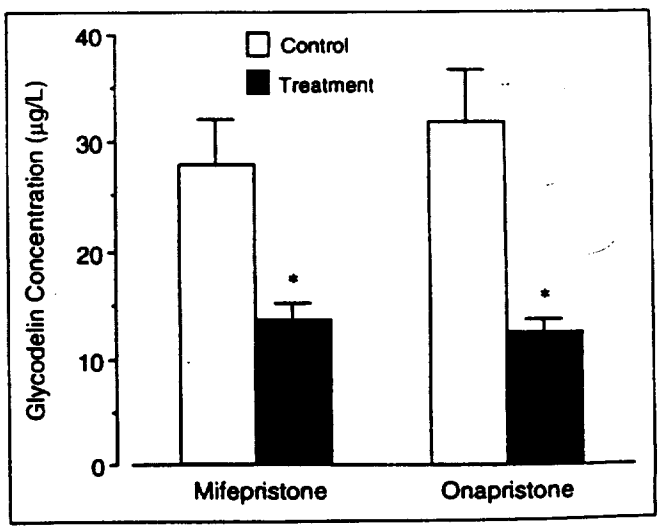

Figure 5 Concentrations of glycodelin measured in serum on day LH+12 of control and treatment cycles. Means ± SEM; *$P < 0.005$.

tory factor significantly enhances the blastocyst formation rates of human embryos in culture systems, suggesting that leukemia inhibitory factor is important for embryo development (20). There also have been reports of lower levels of leukemia inhibitory factor in uterine flushings of women with unexplained infertility compared with those taken from fertile controls (Laird SM, et al., abstract). Thus the reduced glandular immunostaining observed on LH+6 of treatment cycles, a time that corresponds to the beginning of the implantation window, would suggest that treatment with mifepristone and onapristone had affected adversely endometrial receptivity.

The administration of onapristone or mifepristone in the early luteal phase inhibited the appearance of 15-hydroxyprostaglandin dehydrogenase and prevented the down-regulation of estrogen and P receptors in the glands in the midluteal phase of the cycle. The fact that antiprogestins inhibited the increase in leukemia inhibitory factor suggests that the increased immunostaining for leukemia inhibitory factor in the glands during the luteal phase of the normal cycle is because of the action of P. It has been reported that P either does not have (21) or has an inhibitory effect on the production of leukemia inhibitory factor by isolated endometrial epithelial cells cultured in vitro (Laird SM, et al., abstract). Whether these studies using isolated cells in vitro are relevant to the events occurring in the intact tissue in vivo is difficult to determine.

The faint or absent immunostaining for the cell proliferation marker Ki 67 in glands and the occasional stromal staining observed in control-cycle biopsies are consistent with the inhibitory effect of progesterone on proliferation. The increased immunostaining of Ki 67 in treatment cycle biopsies, particularly within the glandular compartment, is in keeping with previous reports of a greater number of mitoses in endometrium after mifepristone has been administered in this way (4). This effect is consistent with progesterone antagonism and retardation of secretory transformation.

As found with onapristone, the effects of postovulatory mifepristone on the endometrium would appear to be long-lasting, because both antiprogestins produced a significant suppression in the serum levels of the endometrial protein glycodelin, measured 10 days after their administration. It is particularly interesting that this effect was observed after treatment with onapristone, in view of the short half-life of this compound and the absence of detectable levels in plasma 4 days after administration of 400 mg (5). This would suggest that the inhibitory effects of both antiprogestins on glycodelin are a consequence of early changes induced in the endometrium soon after their administration.

Although neither antiprogestin affected the length of the luteal phase, significantly greater levels of pregnanediol glucuronide were measured after treatment with mifepristone but not after onapristone. The reason for this is unclear. Concentrations of progesterone and urinary LH have been reported previously to be elevated after early luteal phase administration of mifepristone (3). Although no significant changes in LH were observed in our study, it is possible that an increase in pregnanediol glucuronide could have arisen from an increase in LH pulse frequency as has been described previously following administration of mifepristone in the luteal phase (Critchley HOD and Baird DT, abstract). It is possible that the absence of an effect of onapristone on levels of pregnanediol glucuronide is related to the short half-life of this compound.

Although both antiprogestins have affinity for the glucocorticoid receptor, neither mifepristone nor onapristone exerted significant effect on the pituitary-adrenal axis in the doses used in this study. This is compatible with previous reports (3, 5).

Episodes of bleeding similar to those that occurred shortly after administration of mifepristone and onapristone in this study are well documented and are thought to be because of a direct effect of the antiprogestin on the endometrium (3, 5). It is of interest that this study revealed that endometrium taken in such circumstances is similar both histologically and immunohistochemically to antiprogestin-treated endometrium in which bleeding is not induced. Although this sort of bleeding might affect the usefulness of postovulatory antiprogestins as a regular method of contraception, with the exception of the subject who received onapristone late, these bleeding episodes were very light.

Recent trials have demonstrated the effectiveness of mifepristone for preventing pregnancy when taken in the periovulatory period as either a postcoital or once-a-month contraceptive (22, 23). Previous studies have suggested that such antifertility effects are likely to be a consequence of effects that render the endometrium hostile to implantation. It has been demonstrated that following early luteal phase administration of antiprogestins, the P-dependent down-regulation of estrogen and P receptors is inhibited (3–5) and there is reduced lectin binding in glands, indicating inhibition of secretory transformation. In this present study we have shown that mifepristone and onapristone also have a profound effect on the expression and secretion of endometrial factors that may be of importance for the implantation processes. This adds further insight into the

possible mechanisms by which postovulatory antiprogestins exert their antifertility effects.

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ORIGINAL RESEARCH ARTICLE

ELSEVIER

Effect of Once Weekly Administration of Mifepristone on Ovarian Function in Normal Women

Ximlian Chen and Bilian Xiao

The effects of mifepristone on ovarian function during a once weekly oral administration regimen were studied in nine healthy women. Each received 25 mg mifepristone on cycle days 3, 10, 17, and 24. Ovulation, as documented by hormonal measurements and ultrasonography, was inhibited during treatment in five subjects, with a midcycle surge of luteinizing hormone and ovulation occurring 6–18 days after the last pill was administered in four of the five subjects. These five treatment cycles were prolonged 9–26 days. The other four subjects had normal cycles as judged by serum hormone levels, ultrasonography, and cycle length. All nine subjects had delayed endometrial growth as indicated by ultrasonography. There was a significant correlation between concentrations of serum mifepristone (10 h and 58 h) and α¹-acid glycoprotein, the protein to which mifepristone binds in circulation. Response to mifepristone did not depend on its circulating levels. We conclude that once weekly administration of 25 mg mifepristone can interfere with normal follicular development and function, but the inhibition of ovulation was inconsistent. CONTRACEPTION 1997;56:175–180 © *1997 Elsevier Science Inc. All rights reserved.*

KEY WORDS: mifepristone, ovulation, α_1-acid glycoprotein

Introduction

Mifepristone is well recognized in many countries as an effective drug for termination of early pregnancy. It has other potential uses in fertility control, such as inhibition of ovulation, prevention of implantation, and induction of menstrual bleeding. There have also been attempts to develop mifepristone as a contraceptive agent.[1]

Many regimens involving mifepristone have been studied, including daily, weekly, and monthly administration as well as its use for emergency contraception and in sequential pills for contraception with synthetic progestin. However, all these regimens must be studied further. In view of the long half-life of mifepristone, its effects on the reproductive system in different phases of the menstrual cycle, and its possible acceptability by women, once-a-week administration of mifepristone may be a potentially good contraceptive method.

An intermittent mifepristone regimen has been used to suppress ovulation in monkeys; administration once per week of 25 mg mifepristone orally blocked the expected luteinizing hormone (LH) and follicle-stimulating hormone (FSH) surge, and progesterone levels remained undetectable. Ovulation inhibition was not evident when 12.5 mg mifepristone was administered once weekly.[2] Similar studies in normal women have been reported. Weekly administration of 10 mg mifepristone appears to be at the threshold for suppression of follicular development. Administration of 50 mg mifepristone can inhibit ovulation and corpus luteum function.[3] The present study was designed to determine the effect of mifepristone on ovarian function when administered once-a-week orally to normal women.

In human serum, mifepristone binds specifically to α_1-acid glycoprotein (AAG). The binding of AAG to drugs is of high affinity and low content.[4] Variations in AAG concentration may affect the metabolism and effects of mifepristone in individuals. In this study, we also investigated the serum concentration of AAG in order to determine its relation to mifepristone level and efficacy.

Materials and Methods

Nine volunteer normal Chinese women aged 32 to 40 years (mean age 35.7 ± 2.8 years) were recruited for this study. They had regular menstrual cycles of 26–36 days. Either an intrauterine device or a barrier contraceptive was used for contraception during mifepristone treatment. Each volunteer was carefully counseled and gave informed consent. After admission, a control cycle of daily basal body temperature

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Table 1. Serum concentration of LH, FSH, E_2, P, mifepristone, and AAG

Response	N	LH (IU/L)*	FSH (IU/L)*	E_2 (pmol/L)*	P (nmol/L)	Mifepristone (μg/L)	AAG (mg/L)
Anovulation†	1	6.7	3.76	183.4	1.9	322.8	362.7
Delayed ovulation‡	4	36 ± 27.3	8.1 ± 3.3	458.7 ± 365.1	17.8 ± 12.4	202.8 ± 51.7	349.9 ± 190.1
Normal ovulation‡	4	65.9 ± 37.3	18.4 ± 5.5	549.6 ± 68.1	35.7 ± 15.9	208.1 ± 68.5	406.6 ± 170.5

LH, luteinizing hormone; FSH, follicle-stimulating hormone; E_2, estradiol; P, progesterone; AAG, α-1-acid glycoprotein.
*LH, FSH, E_2, are preovulatory peak values.
†In the anovulation case there was no peak. The mean values of all samples are given.
‡In the delayed ovulation group the peak value occurred on cycle day 30–42, whereas in the normal ovulation group the peak value occurred on cycle day 10–17. Values given in the delayed and normal ovulation group are the means ± SD of four cases in each group.

(BBT) was recorded to rule out an anovulatory cycle. Mifepristone treatment began in the subsequent cycle. Each subject received 25 mg mifepristone (Shanghai Hualian Pharmaceutical Corp., Shanghai, China) orally at 10:00 PM on cycle days 3, 10, 17, and 24. Blood samples were collected every other day at 8:00 AM for one treatment cycle, starting on the second day of the first pill. Serum was separated and stored at −20°C until assay. Follicular growth was monitored by vaginal ultrasonography (Bruel & Kjaer, Denmark). The study was approved by the Ethics Committee of the National Research Institute for Family Planning.

Serum LH and FSH were measured by enzyme immunoassay (EIA). Serum estradiol (E_2) and progesterone (P) were measured by radioimmunoassay (RIA). Reagents and methods for RIA and EIA were supplied by the World Health Organization (WHO). Mean intra-assay coefficients of variation (intra-CV) were 3.52%–6.56% for RIA of E_2 and P, and 1.73%–3.46% for EIA of LH and FSH. Mean interassay coefficients of variations (inter-CV) were 7.42%–11.76% for RIA of E_2 and P, and 7.28%–7.88% for EIA of LH and FSH. Serum levels of mifepristone were determined by HPLC as described by He et al.[5] Serum concentrations of α-1-acid glycoprotein (AAG) were measured by ELISA. ELISA for AAG was modified in our laboratory according to the following procedure. Goat antiserum to human AAG (Shanghai Institute for Biological Product, Shanghai, China) was purified by saturated ammonium sulfate and DEAE-cellulose column to obtain the IgG fraction of the antiserum. Goat IgG to human AAG was conjugated to horseradish peroxidase (Sigma, St. Louis, MO). Microtiter plates (Nunclon, Delta, Denmark) were coated with the purified antiserum to human AAG (titer 5 mg/L) at 4°C overnight and then were washed with phosphate-buffered saline-Tween20 (PBST). The coated plates were successively incubated with standard AAG (cat. no. G9885, Sigma; dilutions 1.56, 3.13, 6.25, 12.5, 25, and 50 μg/L) or serum samples (prediluted 5000-fold in PBST) and antihuman AAG-conjugate (diluted 15,000-fold in PBST). All incubations were for two hours at 37°C. All reagents and samples were added to the wells in 100 μL volumes, and each incubation was followed by washing with PBST. Next the substrate solution (0.4 g/L *o*-phenylenediamine dihydrochloride, 100 μL per well) was added to the wells. After 15 min at 37°C, the reaction was terminated by adding 50 μL of 4N H_2SO_4 per well. The absorbance was measured at 492 nm with Titertek Multiskan (Flow Laboratories, Helsinki, Finland). The intra-CV for standards of low, middle, and high concentration were 2.26%, 3.22%, and 3.58%, respectively. The inter-CV for standards of low, middle, and high concentration were 12.9%, 3.52%, and 4.26%, respectively.

Results

All nine subjects had ovulatory control cycles as judged by BBT. None of the subjects had any untoward effects consequent to the administration of mifepristone. On the basis of serum levels of LH, FSH, E_2, and P, follicular development, and ultrasonography, the ovarian responses to the once-weekly administration of mifepristone manifested three different patterns: anovulation (1 case), delayed ovulation (4 cases), and normal ovulation (4 cases). The data are summarized in Table 1.

Figure 1 depicts the response to mifepristone in the one anovulatory subject. No midcycle LH and FSH surge or ovulatory E_2 and P profile occurred. Ultrasound scanning showed no dominant follicle <12 mm. Her cycle length was 4 days longer than the control cycle.

In the delayed ovulation pattern, four subjects shared some common characteristics: during treatment with mifepristone, the serum LH, FSH, E_2, and P remained at low levels, and there was no LH and FSH surge. During this same period, the maximum follicular diameter was <15 mm. LH and FSH surges, which were proceeded by a rise in E_2 and followed by an elevation in P levels, occurred 6 to 18 days after the last pill of mifepristone in three subjects. In the other subject, there were late elevations in E_2 and P around day 30. Ovulation in all four subjects was evident

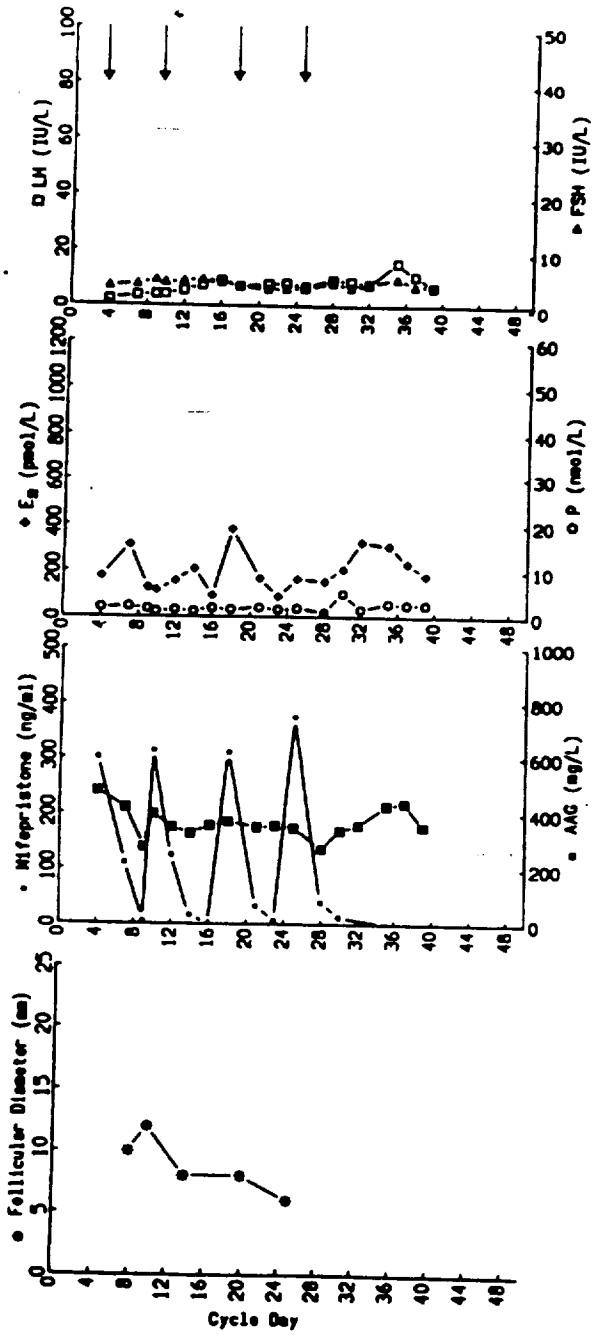

Figure 1. Patterns of serum luteinizing hormone (LH, □), follicle-stimulating hormone (FSH, ▲), estradiol (E_2, ◆), progesterone (P, ○), mifepristone (●), α-1-acid glycoprotein (AAG, ■), and follicular diameter (*) during the treatment cycle in subject 1 receiving 25 mg mifepristone orally on cycle days 3, 10, 17, and 24. ↓ indicates administration of mifepristone. This subject, 32 years old, with an ovulatory control cycle of 34 days, exhibited no ovulation, as indicated by no LH and FSH surge, low levels of E_2 and P, and ultrasonography during mifepristone treatment.

after the last pill by sonogram and BBT. Their cycle lengths were prolonged 9–26 days. Figure 2 shows the response of subject 5, representative of the second pattern in the treatment cycle.

During treatment, four subjects exhibited normal ovulatory cycles. Serum levels of LH, FSH, E_2, and P were all within normal ranges. Both sonogram and BBT indicated ovulation. There was no significant difference between the cycle length in control and treatment cycles. Figure 3 depicts the response of subject 6, representing the normal pattern of ovarian response to the once-weekly administration of mifepristone.

There were no significant differences in serum levels of both AAG and mifepristone among subjects whether ovulation was inhibited, delayed, or normal.

Mifepristone levels in subject 9 were significantly different from the other eight subjects. In subject 9 the concentration of mifepristone increased consistently with a maximal value 106 h after each tablet administration, while in the others the concentration of mifepristone was highest 10 h after drug ingestion. Serum AAG levels correlated with mifepristone levels at 10 h ($r = 0.396$, $p < 0.05$) and 58 h ($r = 0.480$, $p < 0.05$) after drug ingestion in subject 1–8 (Figure 4).

During treatment with mifepristone, the endometrium grew slowly. The thickness of endometrium measured by ultrasonography in the treatment cycle was less than normal. All subjects noted that their menstrual bleeding was less than in normal cycles.

Discussion

In this study, variable responses to 25 mg mifepristone once weekly were observed. Ovulation was inhibited, delayed, or normal in the subjects. The reasons for these differences could be multiple. With a limited dose level, individual variability to the effects of mifepristone could be great.

Mifepristone levels differed markedly among individuals. The circulating levels of mifepristone did not explain the inconsistent inhibition of ovulation. Ovulation was inhibited in some subjects with high mifepristone levels, but in others, ovulation was suppressed with low mifepristone concentrations. Spitz et al. reported that when mifepristone (50 mg/day for 3 days) was administered every 10 days, there was still an endocrine profile compatible with corpus luteum function in the one subject with the highest circulating mifepristone levels.[3]

The three main metabolites of mifepristone, monodemethylated, didemethylated, and hydroxylated metabolites, can bind with the progesterone receptor.[6] In the rat, these metabolites can also terminate

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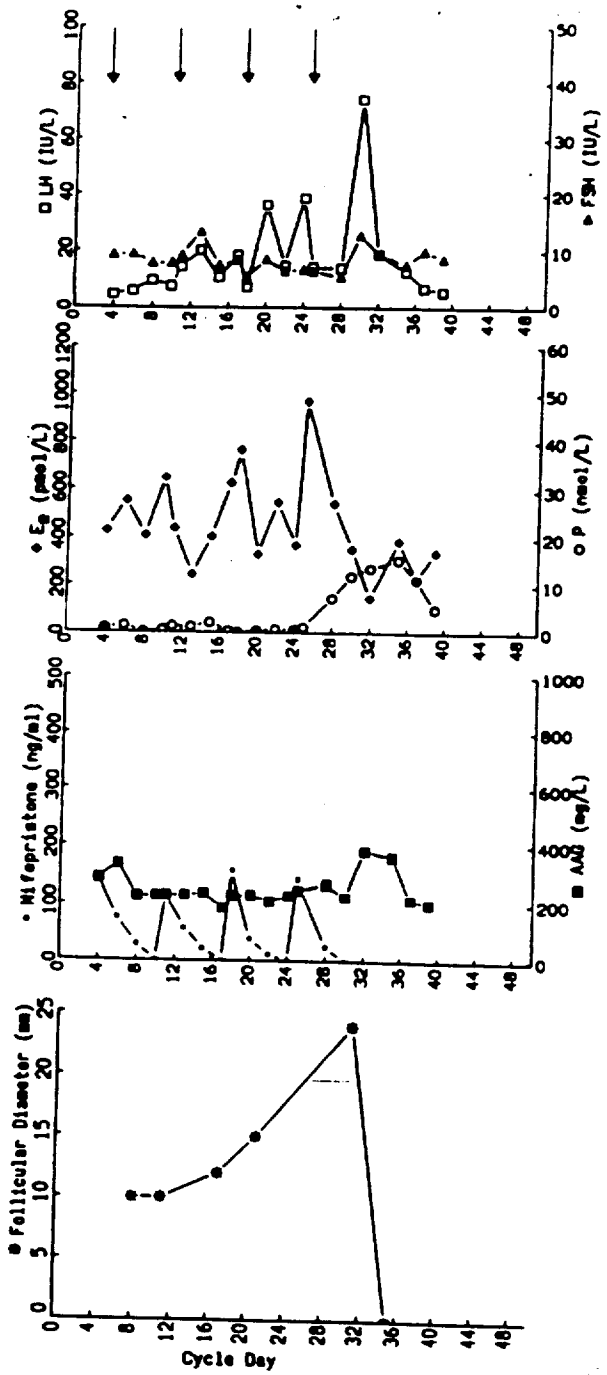

Figure 2. Patterns of serum luteinizing hormone (LH, □), follicle-stimulating hormone (FSH, ▲), estradiol (E_2, ◆), progesterone (P, ○), mifepristone (●), α-1-acid glycoprotein (AAG, ■), and follicular diameter (*) during the treatment cycle in subject 5 receiving 25 mg mifepristone orally on cycle days 3, 10, 17, and 24. ↓ indicates administration of mifepristone. This subject, 36 years old, with an ovulatory control cycle of 30 days, exhibited a delayed ovulation, as indicated by LH and FSH surge, ovulatory levels of E_2 and P, and ultrasonography during mifepristone treatment.

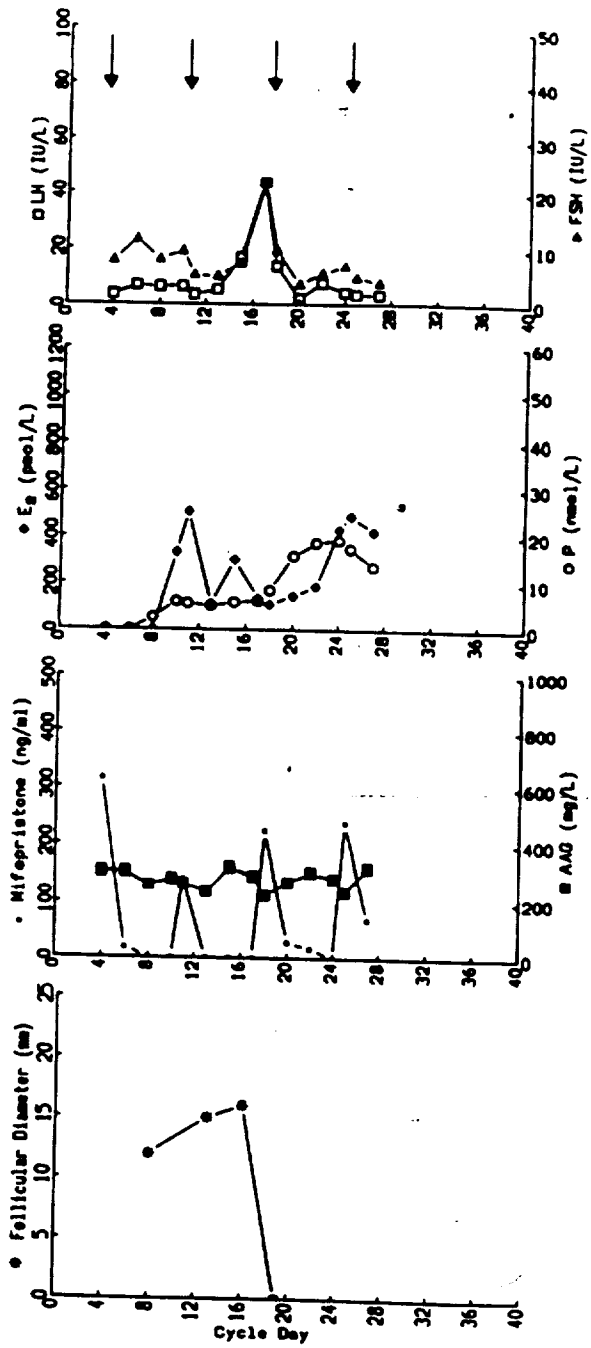

Figure 3. Patterns of serum luteinizing hormone (LH, □), follicle-stimulating hormone (FSH, ▲), estradiol (E_2, ◆), progesterone (P, ○), mifepristone (●), α-1-acid glycoprotein (AAG, ■), and follicular diameter (*) during the treatment cycle in subject 6 receiving 25 mg mifepristone orally on cycle days 3, 10, 17, and 24. ↓ indicates administration of mifepristone. This subject, 36 years old, with an ovulatory control cycle of 28 days, exhibited normal ovulation, as indicated by no LH and FSH surge, ovulatory levels of E_2 and P, and ultrasonography during mifepristone treatment.

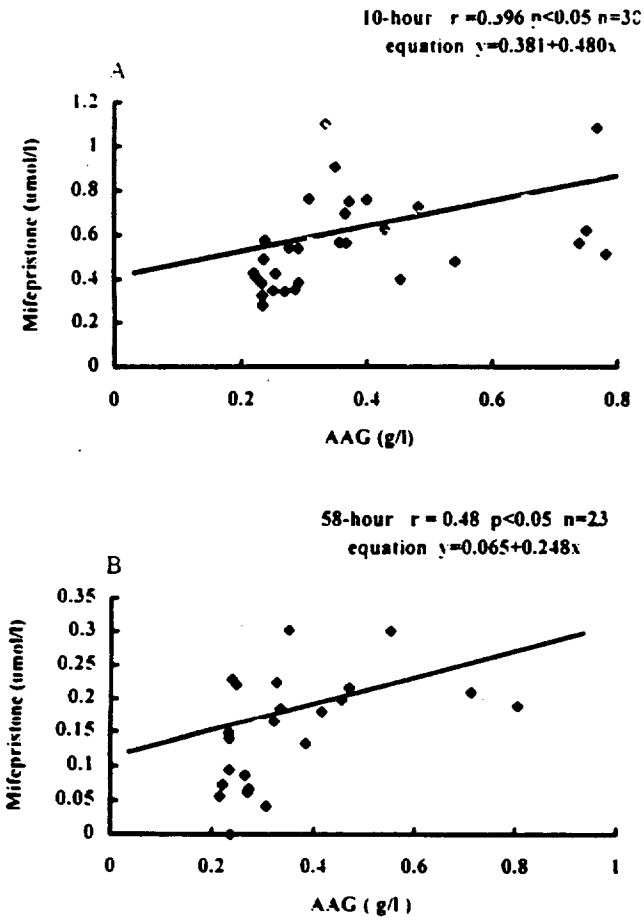

Figure 4. Correlation between serum AAG and mifepristone levels in 8 subjects who received 25 mg mifepristone on cycle days 3, 10, 17, and 24. In panels A and B, mifepristone concentrations were at 10 h and 58 h after the pill intake, respectively.

early pregnancy.[7] Although their effects in the human have not been studied, the metabolites need to be considered because of their high levels in serum and binding with progesterone receptor. It is possible that variations in mifepristone metabolism might account for the variable degree of ovarian suppression. Further studies on the metabolism of mifepristone and the effect of the metabolites may help to elucidate the differences among individuals.

It has been previously demonstrated that as little as 1 mg mifepristone administered daily for 5 days in the preovulatory phase, in the presence of ultrasonic evidence of development of the dominant follicle, will delay or abolish the LH surge and ovulation.[8] Moreover, Ledger et al. have shown that 2 mg mifepristone administered daily can inhibit ovulation successfully.[9] Once-weekly administration of 25 mg mifepristone, as given here, might not maintain effective concentrations continuously. On the other hand, even 50 mg mifepristone for 3 consecutive days administered every 10 days did not uniformly inhibit ovulation.[3]

Individual peak levels of mifepristone at higher single doses, such as 400 mg, 600 mg, and 800 mg, were reported to correlate positively with mean AAG concentrations.[10] This was also shown in our studies with 25 mg mifepristone. Because of the binding characteristics of drugs to AAG, AAG is often the major determinant of variability in plasma protein binding both among individuals and within individuals.[11] The positive correlation of mifepristone with AAG shown in the present study may be one of the factors for differences in mifepristone concentration among individuals.

The present results indicate that once-weekly administration of 25 mg mifepristone is unable to cause consistent inhibition of ovarian function. Progestin-only contraceptives also rely on thickening of cervical mucus, endometrial asynchrony, and possible disturbances of oviductal function in addition to inhibition of ovulation. It has been reported that once-weekly administration of 2.5 mg or 5 mg of mifepristone disturbs endometrial development and the normal decrease in progesterone receptor concentration. Furthermore, the secretory activity of the endometrium is strongly impaired.[12] The mechanism of intermittent treatment with mifepristone needs further study, including the effects on endometrium, cervical mucus, and fallopian tube. The integration of studies on the hypothalamic-pituitary-ovarian axis and its target organs may give a clear view of mifepristone's mechanism of action. Moreover, intermittent administration of mifepristone might still provide effective contraception regardless of is effectiveness via inhibiting ovulation.

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TYPE II (GLUCOCORTICOID) RECEPTORS MEDIATE FAST-FEEDBACK INHIBITION OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS IN MAN

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Summary

The hypothalamic-pituitary-adrenal axis is inhibited via negative feedback, which in rats is mediated via type I and II steroid receptors. Although these receptors are present in neural tissue in man, their respective roles have not been systematically examined. Fast-feedback is the first component of negative feedback, occurs within two hours, and is sensitive to the rate of rising cortisol levels. This study examines the role of type I & II steroid receptors in mediating fast-feedback. A within subjects design was used. Subjects were pre-treated with placebo, spironolactone 200mg or RU486 (mifepristone) - type I & II antagonists respectively. This was followed by infusion of either placebo or hydrocortisone. 8 healthy male volunteers were studied on four separate occasions. Plasma cortisol and ACTH were measured by RIA. Significant elevations of morning basal ACTH and Cortisol following RU 486 relative to placebo or spironolactone were observed. ACTH responses to hydrocortisone (i.e. feedback) were not altered by prior administration of spironolactone. In contrast, RU 486 pre-treatment resulted in a significant attenuation of fast-feedback. These results indicate that type II receptors mediate the fast-feedback phase of negative feedback in man.

Key Words: type II glucocorticoid receptors, hypothalamic-pituitary-adrenal axis, cortisol, ACTH, RU 486

Cortisol, the principal glucocorticoid in man, is essential for life. The physiological effects are both ubiquitous and profound, modulating adaptation to a fluctuating environment, via for example, its effects on intermediary metabolism, immune function in addition to its mineralocorticoid actions on electrolyte and fluid balance (1). As it is integral to the maintenance of cellular function, it is imperative that cortisol is in turn subject to strict regulation. Its release by the hypothalamic-pituitary adrenal axis (HPA axis) is carefully determined by the interaction of three principal controlling mechanisms. Circadian rhythm and response to stress serve to 'drive' the HPA axis to produce cortisol whereas negative

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feedback switches off production and thus maintain homeostasis, the balance between stimulatory and inhibitory influences (2). The importance of over- and under-activity of the HPA can be seen in the association with disease states, classically Cushing's and Addison's disease respectively. Significant perturbations of HPA activity have been identified in other conditions such as major depression (3) and chronic fatigue syndrome (4) and have been postulated to be of pathological significance in these conditions.

The zonae fasiculata and reticularis of the adrenal cortex are the chief sites of cortisol production and secretion which are regulated by ACTH (1). Under the influence of the circadian rhythm, release occurs in bursts whose amplitude is greatest during acrophase at approximately 0800h. This pulsatile pattern is stimulated by corticotropin releasing hormone (CRH) ± arginine vasopressin (AVP) from hypothalamic neurones that receive afferent connections from the suprachiasmatic nucleus. In contrast to peak activity, the nadir is characterised by little CRH/AVP stimulation because removal of the restraining effects of negative feedback by metyrapone blockade of cortisol production, does not result in a surge of ACTH or cortisol (5). Furthermore, this circadian rhythm does not require a functioning negative feedback system as it has been shown to function despite hypocortisolaemia (6). So, these systems controlling HPA activity are independent but continually combine and interact to determine ACTH and cortisol levels.

Negative feedback has a number of different components, distinguished possibly by differences in mechanism and recognised by their time of onset following the elevation of cortisol (7). When suppression of ACTH occurs between minutes and two hours, this is termed fast-feedback. This is sensitive to the rate of rise of cortisol level. Delayed feedback occurs in two phases, early (within two to four hours of increase in cortisol) and late, which occurs after this and these are activated by the peak level of cortisol reached (7).

Feedback systems are mediated via a dual receptor system in rats, sheep, horses and primates with some evidence for such a system in man. This system is comprised of the type I or mineralocorticoid (MCR) receptor and the type II glucocorticoid receptor (GCR) (8). Type I receptors, which have a predominantly septo-hippocampal distribution, have a high affinity but low capacity for cortisol in contrast to the widely distributed type II receptor which has a low affinity but high capacity for binding cortisol. In animal species, these work in concert with the MCR regulating basal circadian output and the GCR controlling peak circadian and stress induced peaks of cortisol. Furthermore there is evidence that it is the GCR, at least in rat, that is responsible for mediating fast-feedback (9).

To date, there has been little data from human studies evaluating the respective contributions of the MCR and GCR in regulating the HPA axis. The aims of this study are twofold. Firstly, to examine the relative contribution of these receptors to the basal circadian peak of both ACTH and cortisol production and secondly, to examine their respective roles in mediating the fast-feedback phase of autoregulation of HPA activity. This was achieved using the selective antagonists spironolactone (SPI)(which acts at type I receptors) and RU486 or mifepristone (MIF), a selective type II antagonist (10, 11).

Methods

Eight healthy male volunteers, aged 23 - 38 years and of normal body weight (69 - 94kg) were recruited from staff and students of the Department of Psychological Medicine. All were

free of physical illness and in particular had no history of endocrinopathy or psychiatric disorder. None had a history of drug or alcohol abuse. Prior to the studies, a physical examination was performed in addition to baseline estimation of full blood count, urea and electrolytes, liver and thyroid function tests. Informed consent was obtained, and approval from the local ethics committee.

Each subject was tested on four separate occasions at least one week apart over an eight week period. Studies were performed in a single blind, randomised order. At 2300h, the night before tests days, subjects were administered either placebo (PLA), RU486 400mg or spironolactone 200mg. On each test day, subjects were kept fasting from midnight and had a 22G cannula inserted into forearm veins bilaterally at 0830h, and sealed with a rubber bung. These were kept patent using a heparin / saline solution. All were required to remain recumbent throughout the test period. Infusions of either hydrocortisone (Solu-Cortef, hydrocortisone as the sodium succinate, Upjohn Ltd, Crawley, UK) $5\mu g.kg^{-1}.min^{-1}$ in 0.9% normal saline or an equal volume of 0.9% normal saline were administered. The infusions were given over a 60 min period between 0900 and 1000h using a metered syringe pump (IVAC P2000, Wellmed Ltd, Hampshire, UK). The four conditions were (1) placebo + placebo (2) placebo + hydrocortisone (3) spironolactone + hydrocortisone (4) RU486 + hydrocortisone.

Blood samples were taken from the arm contralateral to the infusion at -15min (0845), 0, +15, +30, +45, +60, +90, +120, +150, +180. Eight ml of blood was taken at each time point with immediate division of 4ml to an EDTA containing tube and 4mls to a tube with no anticoagulant. Blood was immediately centrifuged and stored at -70°C.

Plasma cortisol and ACTH were analysed in batch blind to subject status. Cortisol was measured with a radioimmunoassay (Cunnah et al, 1987). ACTH was measured using a commercially available IRMA (Nichol Institute, Ca. USA) with a detection limit for plasma ACTH of $< 0.44 pmol.l^{-1}$. The inter- and intrassay coefficients of variation were $< 5\%$ across the working range of the assay ($0.44 - 308 pmol.l^{-1}$).

Data were analysed by means of Statgraphics, version 7 (Statistical Graphics Corporation, 1993). Baseline cortisol and ACTH values were calculated as the mean of -15 and 0 min samples. 1-way and two-way analysis of variance (ANOVA) with appropriate post-hoc comparisons were used where appropriate. ΔACTH was calculated as the maximal deviation from placebo values, between 0 and +90mins as this is the period that cortisol levels were rising in response to hydrocortisone infusion.

Results

Mean basal concentrations of ACTH (at 0900h) varied significantly between the treatment groups ($F(2,21) = 22.70$; $p < 0.0001$). Mean (± SEM) basal levels for each treatment group were $7.79 \pm 1.19 pmol.l^{-1}$ for PLA; $7.30 \pm 0.72 pmol.l^{-1}$ for SPI; $18.39 \pm 2.26 pmol.l^{-1}$ for RU486. Post hoc testing demonstrates that RU486 significantly elevates morning ACTH levels ($p < 0.05$) in contrast to spironolactone which has no impact (see Fig. 1a).

Similarly, one-way analysis of variance of mean basal cortisol levels revealed a significant difference amongst the groups ($F(2,21) = 13.15$; $p = 0.0002$). Mean basal cortisol levels were $488 \pm 38.7 nmol.l^{-1}$ for the PLA group; $460 \pm 23.59 nmol.l^{-1}$ for SPI and 650 ±

38.11nmol.l^{-1} for HC + RU486 respectively. Post-hoc testing demonstrated that baseline cortisol levels in the group administered RU486 were significantly greater than those observed in the three other treatment conditions ($p < 0.05$) (see Fig. 1b).

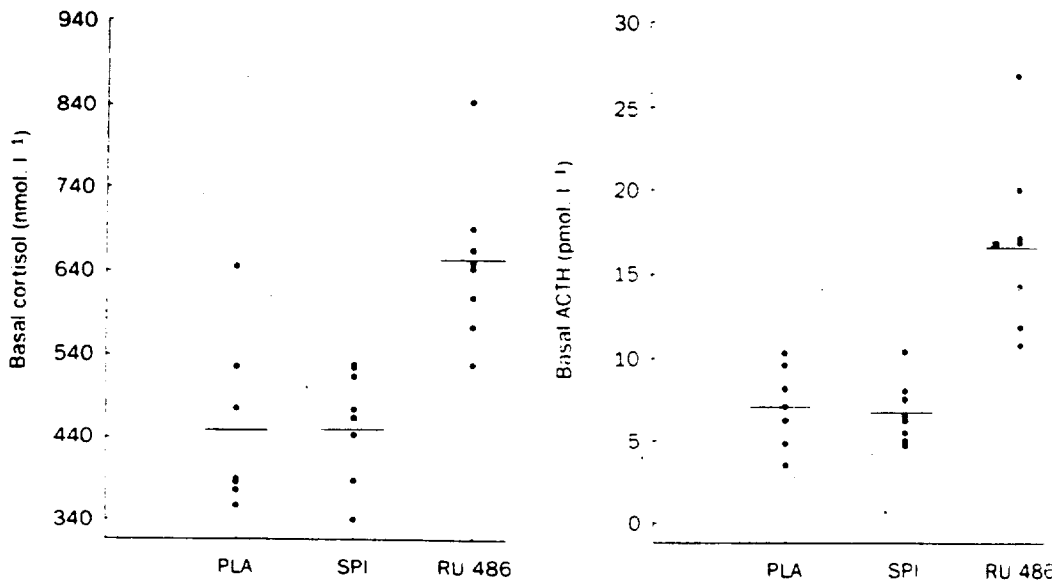

Fig.1

(a) Basal cortisol values in nmol.l^{-1}. Individual values represented (± SEM) comparing the effects of placebo, spironolactone and RU 486. Basal values calculated as the mean of samples obtained at 0845 and 0900. (b) Basal ACTH values in pmol.l^{-1} Individual values represented (± SEM) comparing the effect of placebo, spironolactone and RU 486. Basal values calculated as the mean of samples obtained at 0845 and 0900.

Hydrocortisone infusion resulted in an increase in cortisol levels in the three groups where it was administered. Peak levels occured at +60 to +90 in all cases. Δcortisol (maximal displacement of cortisol from baseline) did not vary significantly between the groups ($F(2,21) = 2.54$; $p = 0.1$) (see fig. 2a)

The drop in ACTH following HC infusion (ie feedback) is similar with placebo and spironolactone pretreatment. RU486 markedly attenuates the drop in ACTH. The mean ± SEM ΔACTH responses to HC (calculated relative to placebo) were as follows: PLA pre-treatment -3.02 ± 0.33pmol.l^{-1}, SPI pre-treatment -3.41 ± 0.46pmol.l^{-1}, RU486 pre-treatment 9.95 ± 1.56pmol.l^{-1} (see Fig. 2b). A 2-way repeated measures ANOVA reveals a significant group x time interaction ($F(9,311) = 2.293$; $p = 0.0005$). When baseline ACTH is entered as a covariate, this interaction remains significant. Post-hoc analysis reveals a significant difference in ACTH responses between the PLA + PLA/RU486 + HC and the PLA + HC/SPI + HC groups ($p < 0.05$). Mifepristone is seen to significantly attenuate the fast-feedback response.

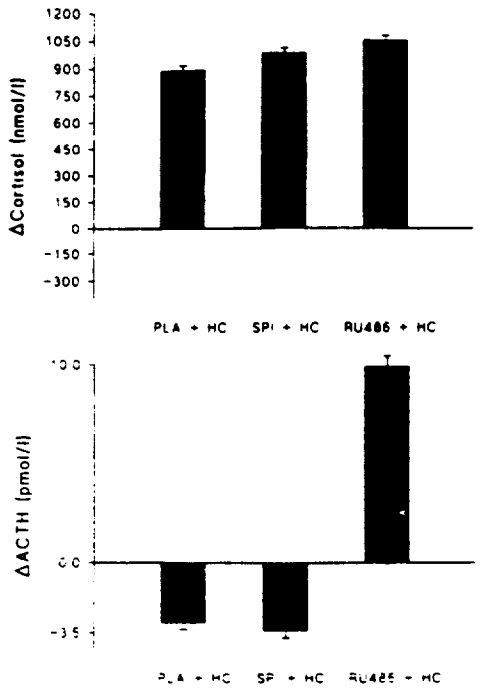

Fig. 2

(a) Δcortisol responses following hydrocortisone 5μg.kg.min^{-1} infusion. Comparison of mean Δcortisol values show no significant differences in the three groups. (b) ΔACTH responses calculated relative to placebo pre-treatment and placebo infusion. ΔACTH following RU486 is significantly greater than ΔACTH for placebo or spironolactone pre-treatment, in response to standardised hydrocortisone infusion.

Discussion

This study shows that pretreatment with the type II glucorticoid receptor antagonist RU486, causes a significant elevation of the basal ACTH and CORT levels in a group of healthy male volunteers. This is in contrast to the effects of spironolactone which does not alter basal ACTH or CORT levels. Furthermore, using a standardised HC infusion challenge, suppression of ACTH occurred which was not significantly altered by pretreatment with spironolactone. In contrast to this finding, pretreatment with RU486 resulted in abolition of ACTH suppression by hydrocortisone infusion, with levels of ACTH comparable to those seen with placebo infusion alone.

Elevated levels of ACTH and CORT occur in response to administration of RU486 at a dose of 400mg. This is the dose required for antagonism of glucocorticoid receptors and its antiglucocorticoid effect to occur - it is known that the antiprogesterone effect occurs in response to much lower levels (10). This increase in morning ACTH and CORT in man is in agreement with Gaillard et al (1984)(12), who showed that mifepristone elevated basal

levels of CORT at the peak of circadian rhythm but with little effect on trough levels. This elevation of basal ACTH and cortisol been further demonstrated in a group of healthy volunteers administered 200mg of RU486 daily for a period of eight days (13). Administration of spironolactone, in contrast, failed to alter basal levels of these suggesting that it is type II and not type I receptors that are of importance in limiting circadian peak levels of ACTH and CORT in man.

The relevance of this finding is twofold. Firstly, it demonstrates that an antiglucocorticoid effect has been achieved (14). Secondly, it is consistent with a large animal literature in which the type II glucocorticoid receptor serves to attenuate peak activity of the hypothalamic-pituitary-adrenal axis (15). It is generally accepted that circadian trough and peak cortisol levels are determined principally by the type I and II steroid receptors respectively, with type I occupancy a prerequisite for functioning of the type II receptors (16).

Plasma levels of ACTH and cortisol are determined by a dynamic regulatory system. Stimulation of their release occurs in response to the circadian drive and stress whereas their release is inhibited by negative feedback. Administration of RU486 blocks the type II receptor and removes negative feedback allowing escape of unopposed circadian driven ACTH and cortisol levels. As conditions for the procedure were standardised, it is unlikely that this consistent finding has occurred as a result of stimulation from an exogenous stressor. As these elevations of peak ACTH and CORT levels were seen 10h following RU486 administration, this indicates an effect mediated by the delayed phase (7) of negative feedback is responsible for regulating the peak of the circadian cycle.

Hydrocortisone infusion resulted in a significant elevation of cortisol levels in the three groups that it was administered. Moreover, under the standardised testing procedures, the elevations of cortisol were similar across the three groups meaning that the stimulus for fast feedback was standardised. The impact of these consistent rises in cortisol was to produce a fall in ACTH levels within 15 - 30 minutes of the start of the hydrocortisone infusion. This fastfeedback effect was not altered by prior administration of the type I receptor antagonist spironolactone as the fall in ΔACTH observed under these conditions was similar to that seen following placebo pretreatment. This suggests that the type I receptor is not involved in mediating fast-feedback which accords with the animal literature (16,17). Alternatively, it may indicate that spironolactone has failed to block the type I receptor. This is unlikely as it has repeatedly demonstrated in vivo potency as an antagonist at the mineralocorticoid receptor (11).

In contrast to this, prior administration of RU486 does significantly alter the fastfeedback response. ΔACTH responses are clearly distinguishable from those seen in response to hydrocortisone infusion alone or in combination with spironolactone pre-treatment. As baseline ACTH levels were significantly elevated relative to the other treatment conditions, these baseline values were entered as covariates and the differences remained significant as they did when group reponses over time were examined. This finding is most plausibly interpreted as that the effect of RU486 is to negate the influence of HC infusion and fast-feedback. The fall in ACTH levels that occurs over time is consistent with the diminishing drive to the HPA from the endogenous circadian rhythm which occurs following the 0800h peak of stimulation (18). If this were fast-feedback, it might be expected to precipitously reduce ACTH output in the face of such an elevation of cortisol. Given that the half-life of ACTH is approximately 15 minutes, removal of drive by a massive increase in negative

feedback would result in a rate of decline of ACTH of the order of 50% every 15mins, which is not seen.

In summary, administration of the type II receptor antagonist RU486 causes an elevation in basal levels of ACTH and cortisol - an effect not seen following type I receptor blockade with spironolactone and which is dependant on the delayed phase of negative feedback. Hydrocortisone infusion at a rate of $5\mu g.kg.min^{-1}$, produced a significant inhibition of ACTH release which was not altered by pre-treatment with spironolactone. In contrast to this, RU486 pre-treatment was able to block the feedback inhibition caused by hydrocortisone infusion to levels comparable to those seen with infusion of placebo. This is evidence in favour of the dual receptor model of regulation of negative feedback. Furthermore, it indicates that type II receptors are responsible for mediating the effects of negative feedback.

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ORIGINAL RESEARCH ARTICLE

ELSEVIER

Effects of a Sequential Regimen of Mifepristone-Medroxyprogesterone Acetate on Ovarian Function, Endometrial Development and Hormonal Parameters

H.B. Croxatto, M.R. Massai, A.M. Salvatierra, B. Fuentealba, H.D. Croxatto, and P. Lähteenmäki*

The efficacy of a low dose of mifepristone, 5 mg/day for the first 15 days of the menstrual cycle, followed by medroxyprogesterone acetate (MPA), 10 mg/day for the next 13 days, for inhibiting ovulation was assessed in ten volunteers who were treated for three successive cycles. Hormonal determinations in blood and urine samples, ovarian ultrasonography and an endometrial biopsy taken on day 21–24 of the third treatment cycle were used to monitor the cycles. Ovulation was confirmed in 11 of the 30 treated cycles and, in these 11, the LH peak and follicular rupture occurred during MPA treatment periods. Out of 19 anovulatory cycles, 16 had no increase in progesterone levels and another 3 developed a luteinized unruptured follicle. Progestin administration induced secretory changes in the endometrium, but irregular or delayed development was found. Regular withdrawal bleeding occurred in all subjects. These data indicate that the sequential regimen can suppress ovulation while maintaining regular bleeding but increased efficacy is needed for phase II clinical trials. CONTRACEPTION 1996;54:79–86

KEY WORDS: mifepristone, RU486, antiprogestin, ovulation inhibition, progestin, endometrium, bleeding pattern

Introduction

It has been demonstrated that mifepristone administration during the follicular phase alters follicular growth, inhibiting ovulation.[1–4] This effect makes possible the development of an estrogen-free oral contraceptive that suppresses ovulation. However, the long-term continuous or intermittent administration of antiprogestins would expose women to unopposed estrogen, with the consequent unwanted effects upon the endometrium and the bleeding pattern. To avoid this problem a sequential antiprogestin-progestin regimen has been proposed.[2] In theory, the antiprogestin given during the first half of the cycle would prevent ovulation in that period and the progestin, given during the second half of the cycle, would act to keep ovulation suppressed and to transform the endometrium allowing cyclic bleeding upon its withdrawal.

Several schemes of treatment have been tested.[5–7] In the first two studies, the antiprogestin was given in a dose of 25 mg/day for the first 14 or 21 days of the menstrual cycle, followed by the administration of a synthetic progestin, norethisterone or medroxyprogesterone acetate (MPA), 5 mg/day, for the next 10 days. These schemes were tested for a single cycle or for three consecutive cycles with a 5-days pill-free interval between cycles. Although they allowed regular bleeding, they were only partially effective for inhibiting ovulation, as assessed by the hormone levels in plasma.

In a different scheme, higher doses of mifepristone, 50 mg/day for 3 days, and MPA, 10 mg/day for 10 days, were combined in an intermittent regimen. The antiprogestin was given during the later stages of follicular development (days 9 to 11) to "knock out" the leading follicle and again at the end of the progestin treatment period (days 27 to 29), to reinforce progestin withdrawal.[7] With this regimen, the proportion of biphasic cycles, 12 out of 32 cycles, was similar to that observed in the previous studies, but follicular rupture was confirmed, by the ultrasound profile of the leading follicle, in only one of those twelve cycles. This suggested that the efficacy of these sequential regimens for inhibiting ovulation could be higher

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than that previously reported and justifies careful monitoring of the leading follicle in this type of trials.

In the present study, we investigated the effectiveness of a simplified antiprogestin-progestin sequential regimen for inhibiting ovulation. Since in previous work it was shown that a daily dose of 5 mg mifepristone for 30 days was effective in preventing ovulation during treatment in all of eleven treated women,[3,4] we choose to test the effects of this low dose for the first 15 days of the menstrual cycle, followed by MPA, 10 mg/day for the next 13 days. The complete sequence was repeated for three successive cycles without leaving resting days between treatment cycles.

Materials and Methods

Ten healthy women, surgically sterilized, regularly cycling, mean age 36.6 years (range 30–40) and mean weight 58.6 kg (range 48.5–69.5) volunteered for the study. The study was approved by the Institutional Ethics Committee and subjects were admitted after giving their informed consent.

This was an open, non-randomized, phase I clinical study, in which each volunteer was her own control. Each subject was studied for one baseline cycle, three consecutive 28-day periods of treatment and one post-treatment cycle.

During each period of treatment, each volunteer received mifepristone (RU486, Roussel-Uclaf, Romainville, France), 5 mg/day, on days 1 to 15 of the cycle and medroxyprogesterone acetate (MPA), 10 mg/day, on days 16 to 28 of the cycle. The entire sequence was reinitiated on the day following the last MPA pill, regardless of the occurrence of menses.

Each subject kept a record of pill ingestion time, bleeding, spotting, any symptoms, concurrent illness and other drug intake. Hematology and serum chemical analysis (serum glutamic oxalacetic and pyruvic transaminase, lactic dehydrogenase, alkaline phosphatase, bilirubin, total protein, cholesterol, uric acid, urea nitrogen, glucose, inorganic phosphate and calcium) were performed at admission, at the end of treatment and after the post-treatment cycle. Blood samples were taken twice a week throughout the study from each volunteer to determine estradiol and progesterone concentrations. A daily first morning urine sample was collected for measuring LH concentration. Additional blood samples were taken to determine mifepristone levels in plasma during the second cycle of treatment. Blood samples were taken on day 1, just before (0 h) and 1 hour after the first mifepristone pill intake and on day 15 of treatment, 24 h after the preceding pill (0 h) and 1 h after mifepristone pill intake. An additional sample was taken 72 h after the last mifepristone pill intake to check for wash out.

Ovarian and uterine echography were performed, three times a week throughout the study, to assess follicular growth and endometrial thickness, using an Aloka SS D 620 ultrasound system, 5 MHz, with a vaginal probe. An endometrial biopsy was taken in the third cycle of treatment, 6 to 9 days after the onset of MPA treatment, to assess endometrial histology according to the criteria of Noyes or Maqueo.[8,9]

Hormones in plasma and LH in urine were measured according to the procedures and with the reagents supplied by the World Health Organization. The lower limits of sensitivity for estradiol, progesterone and LH assays were 70 pmol/L, 1.2 nmol/L and 1.2 IU/L, respectively. The intra-assay coefficient of variation of low, medium and high pool, for E2, P and LH ranged from 9–15%, 5–20% and 5–10%, respectively. The inter-assay coefficient of variation ranged from 12–25% and from 7–11% for steroid hormones and LH, respectively.

Mifepristone levels in plasma were determined at the Steroid Research Laboratory, University of Helsinki, Finland, using previously described methods.[10] The intra- and inter-assay coefficients of variation were 9.3% and 13–16%, respectively.

Previous studies have shown that administration of mifepristone (or antiprogestins) in the follicular phase can be associated with lower estrogen levels in serum when follicular growth is halted[1,4,11] or, on the contrary, it can increase estrogen levels when a mature follicle that is prevented from ovulating keeps on growing,[12] and can also cause follicular luteinization without rupture.[7,13] Because it was of interest to assess the occurrence of these conditions during this treatment, the following working definitions were used in the analysis of the data:

Hypo-, normo- and hyperestrogenic cycles were defined by plasma E2 concentration under 400 pmol/L throughout the follicular phase, between 400 pmol/L and 1500 pmol/L throughout the follicular phase, and over 1500 pmol/L in two or more samples taken during the follicular or luteal phase, respectively.

Follicular rupture: abrupt disappearance of a follicular echo-image larger than 16 mm. Ovulation: follicular rupture followed by increased plasma progesterone levels over 12 nmol/L in at least two consecutive samples. Luteinized unruptured follicle (LUF): persistent echo-image of a follicle concomitant with increased plasma progesterone levels over 12 nmol/L in one or more samples.

In the statistical analysis, the Student's t-test was used to determine differences in estrogen levels during equivalent periods of time between the baseline and the first treated cycle, in the length of the cycles

and of the phases, and in progesterone levels of biphasic cycles. Analysis of variance was used to compare endometrial thickness and the length of the cycle during the study. Dunnett test was used when analysis of variance reached significance. Normal distribution of data, on plasma RU486 levels and the onset of bleeding, was assessed using the Shapiro-Wilk test. Wilcoxon signed rank test followed by the Dunn test, was used in the analysis of the onset of bleeding. A value of $p < 0.05$ was considered statistically significant.

Results

All baseline cycles were ovulatory and showed normal ovarian function, defined by the finding of an LH peak in urine, estrogen and progesterone oscillations, follicular growth and length of the phases within normal limits.

Treatment inhibited ovulation during the three treatment cycles in 5 women. The regimen was partially effective in 3 women and totally ineffective in another 2 women. During treatment, 11 out of 30 cycles corresponded to ovulatory cycles and the other 19 cycles were anovulatory (Table 1).

According to whether or not ovulation occurred and the hormonal parameters, three types of cycles were observed during treatment: biphasic ovulatory, biphasic anovulatory and monophasic anovulatory.

Ovulatory cycles differed from baseline cycles. The rate of follicular growth was slower and in one case the follicle reached a diameter of 28 mm before rupture. In these cycles, the LH surge always occurred during the MPA treatment period, and was delayed 4–8 days in comparison with the baseline cycles (Figure 1). This is reflected in increased length of the follicular phase (shown in Table 4).

Among the anovulatory cycles, 3 cycles presented a biphasic hormonal profile. In these three cycles the luteal phase progesterone levels were much lower than in baseline cycles and they were associated with unruptured follicles (Figure 2). The other 16 cycles had a monophasic hormonal profile, with no increase in progesterone levels in spite of a delayed rise in LH level (Figure 3).

Table 1. Efficacy of the RU486-MPA sequential regimen for inhibiting ovulation

Cycle	Anovulatory/Total N
Baseline	0/10
Treatment	
First	5/10
Second	6/10
Third	8/10
Post-treatment	4/9

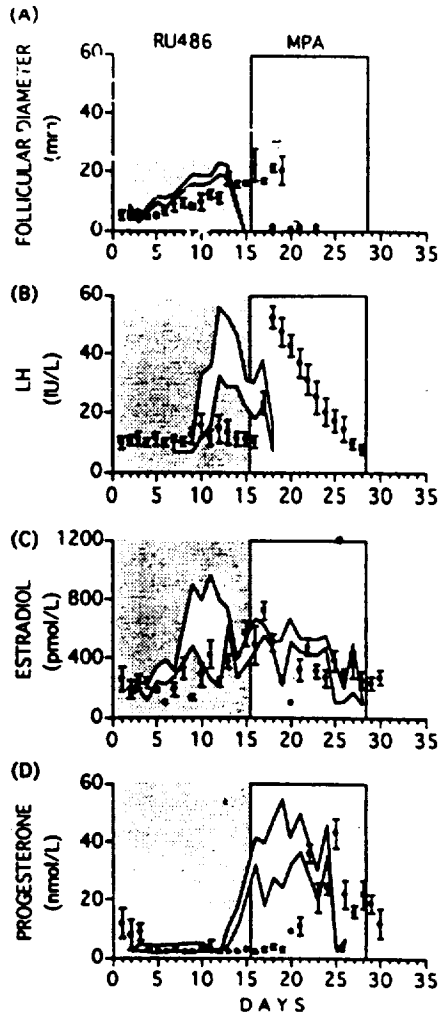

Figure 1. Follicular and endocrine profile of ovulatory cycles during treatment with a sequential regimen of RU486 mg/d and MPA 10 mg/d. Follicular growth (A), LH (B), estradiol (C) and progesterone (D) plasma levels during treatment cycles. Dots and bars represent the mean ± SEM of 11 treated cycles. Compare with the area encompassed between the two continuous lines which represents ± one SEM of values obtained from 10 baseline cycles.

The echographic pattern of follicular growth showed an enlarged follicle (mean: 41.3 and range: 28–70 mm in diameter) in 14 cycles, in 8 of the 10 women during treatment. Only 4 of these 14 follicles were associated with high plasma estradiol levels (1500–3000 pmol/L). Three additional enlarged follicles were observed during the post-treatment cycle (Table 2). Resolution of all enlarged follicles occurred spontaneously.

Hormonal Parameters

Maximal plasma E2 levels, during the periods of treatment with mifepristone, were not different between

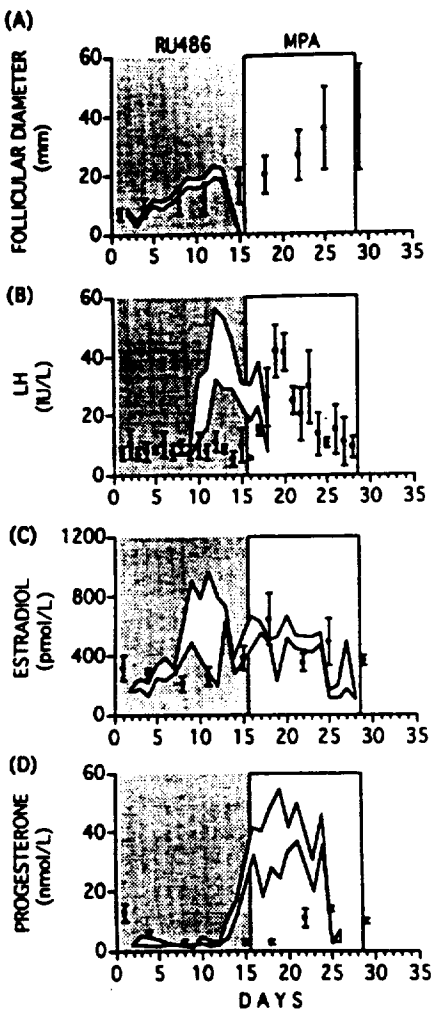

Figure 2. Follicular growth (A), plasma LH (B), estradiol (C) and progesterone (D) levels in biphasic cycles with luteinized unruptured follicles during treatment with RU486-MPA sequential regimen. Dots and bars represent the mean ± SEM of 3 treated cycles. Compare with the area encompassed between the two continuous lines which represents ± one SEM of values obtained from 10 baseline cycles.

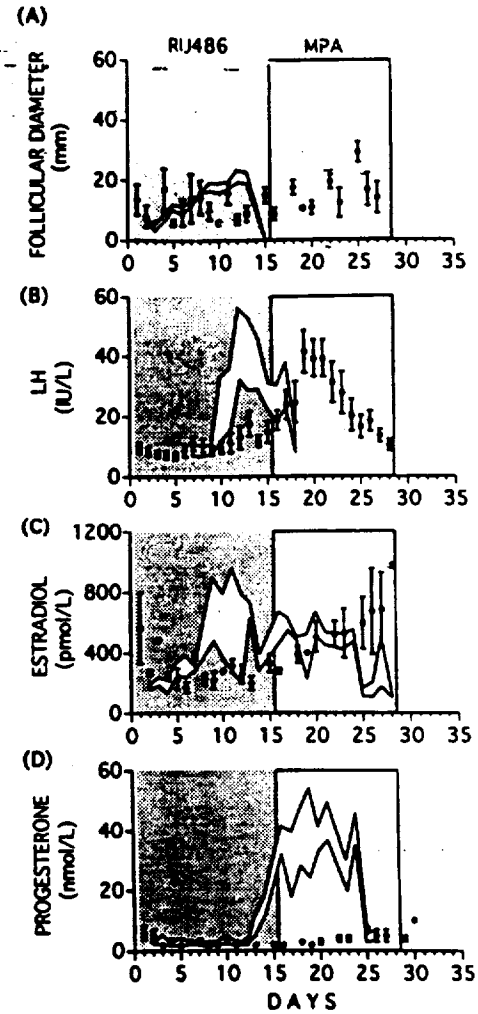

Figure 3. Follicular growth (A), plasma LH (B), estradiol (C) and progesterone (D) levels in monophasic cycles during treatment with a sequential regimen of RU486-MPA. Dots and bars represent the mean ± SEM of 16 treated cycles. Compare with the area encompassed between the two continuous lines which represents ± one SEM of values obtained from 10 baseline cycles.

the three treated cycles (ANOVA), but those of the first treatment cycle were lower than in the first 15 days of the baseline cycle (X ± SEM: 454.7 ± 80 vs. 730.8 ± 94.6 pmol/L, p = 0.037 Student's t-test). Maximal E2 levels in the second and third mifepristone treatment periods were 605.6 ± 130.5 and 596.1 ± 264.7 pmol/L, respectively. During the MPA treatment periods, maximal E2 levels were not different from those of the baseline cycle but increased E2 levels, over 1500 pmol/L, were observed in four monophasic cycles. In two cycles, a fall of these E2 levels was observed after the first mifepristone pill of the next treatment period. In the other two cycles, in which the MPA period was not followed by mifepristone intake, E2 levels increased over that observed at the end of the third treatment cycle, remaining high for several days (Figure 4).

An increase in urinary LH concentration was detected in 29 out of 30 treated cycles. In all of them, the highest LH levels occurred during the MPA treatment period. In contrast, attenuated or no LH peaks were detected during the mifepristone treatment period.

As shown in Figure 5, plasma progesterone levels attained during the luteal phase of biphasic cycles decreased in successive cycles (ANOVA, p = 0.005)

Table 2. Occurrence of enlarged follicles under RU486-MPA sequential regimen*

	No. of Cycles				
		Treatment			
Type of Cycle	Baseline	1st	2nd	3rd	Post-treatment
Ovulatory	0	1	0	0	0
Biphasic with LUF**	0	0	1	1	3
Monophasic hyperestrogenic§	0	1	1	2	0
Monophasic normoestrogenic¶	0	2	1	4	0
Enlarged/total cycles	0/10	4/10	3/10	7/10	3/9

*Follicles with a mean diameter larger than 25 mm. Those persisting into the next cycle were counted only once and assigned to the menstrual cycle in which they were first seen.
**Luteinized unruptured follicle.
§Maximal plasma E_2 levels throughout the cycle over 1500 pmol/L; progesterone, under 12 nmol/L.
¶Maximal plasma E_2 levels throughout the cycle between 400 pmol/L and 1500 pmol/L; progesterone, under 12 nmol/L.

being in the second and third cycle, significantly lower than in the baseline (Dunnett test $p < 0.05$).

Increased plasma mifepristone concentration was observed 1 h after pill intake in all women (X ± SEM: 585.2 ± 97.6 nmol/L). As shown in Figure 6, values did not distribute normally ($p = 0.0369$ Shapiro-Wilk) and large differences were observed among subjects. Three women had values between 122–176 nmol/L and the other seven had values between 631–962 nmol/L. After a 72 h wash out period, mean mifepristone concentration decreased to 73.7 ± 15.8 nmol/L (X ± SEM).

Effects on the Bleeding Pattern and Length of the Cycle

The effect of treatment on withdrawal bleeding is shown in Table 3. By design, in the first two treatment periods, progestin withdrawal was likely to be potentiated by the ensuing treatment with mifepristone but at the end of the third treatment cycle, bleeding should occur by progestin withdrawal only. After the first two treatment cycles, withdrawal bleeding started usually within the first three days following the last MPA pill (range: -2 to 4 days and 2 to 5 days for the first and second cycle, respectively). At the end of the third cycle of treatment, the onset of withdrawal bleeding was significantly delayed (range: 1 to 28 days) with respect to the previous cycles ($p = 0.0197$, Wilcoxon test; $p < 0.05$, Dunn test).

The duration of the bleeding episodes after each treatment cycle was not significantly different from that of the baseline and post-treatment cycles.

Cycle length of all cycles is shown in Table 3 and of ovulatory cycles only in Table 4. Treated cycles showed a tendency to be slightly longer than baseline in all women. Twenty-eight of the 30 treatment cycles were 0–10 days longer than their corresponding baseline cycles, with a median of 3.5 days in the first, 2 days in the second and 3 days in the third cycle of treatment. One cycle was 6 days shorter and another, 25 days longer than the baseline.

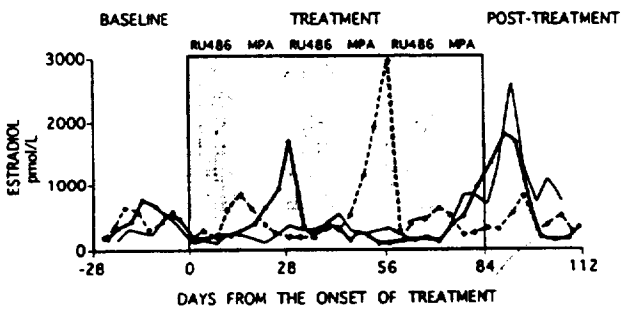

Figure 4. Estradiol profile of 3 subjects with estradiol levels over 1500 pmol/L before, during and after the RU486-MPA sequential regimen. One subject presented high estradiol levels twice during the study. Note immediate reduction in E2 levels after the first RU486 pill intake in treatment cycles 2 and 3 in contrast to more persisting high levels after the last MPA course not followed by RU486.

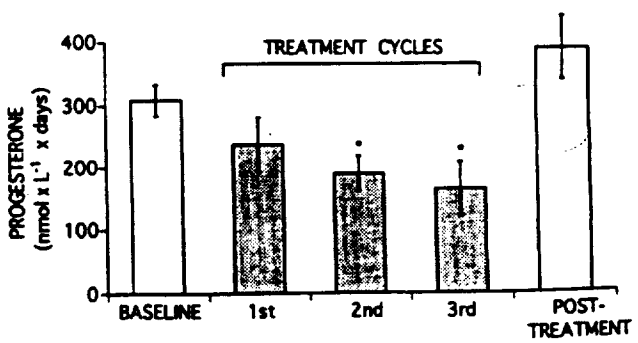

Figure 5. Plasma progesterone levels in baseline, treatment and post-treatment biphasic cycles. Values are the mean ± SEM of the area under the curve calculated for the luteal phase of each subject. Treatment consisted in a sequential regimen of RU486 5 mg/d and MPA 10 mg/d. *Significantly different from baseline ($p < 0.05$ Dunnett test).

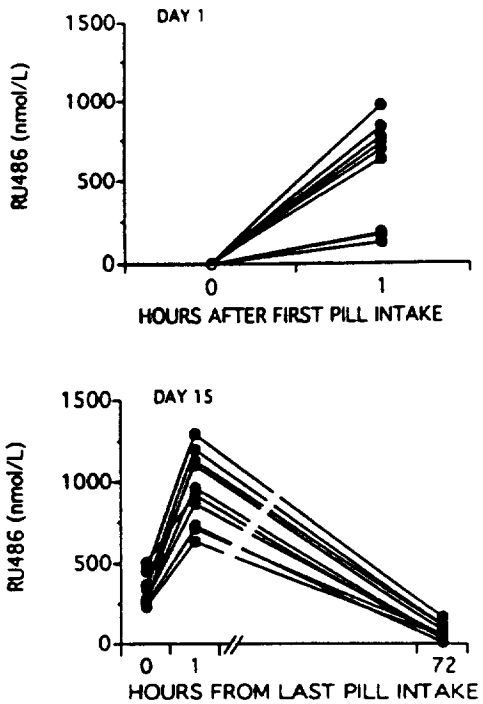

Figure 6. Plasma RU486 levels attained just before and 1 h after the first RU486 pill intake on day 1 (upper panel) and just before and 1 h and 72 h after the last RU486 pill intake on day 15 of the second cycle of treatment (lower panel). Treatment consisted in a sequential regimen of RU486 5 mg/d and MPA 10 mg/d.

The length of the follicular and luteal phase was calculated for the ovulatory cycles (Table 4). In these cases, treated cycles were longer than the baseline cycles, due to prolongation of the follicular phase. No significant changes in the length of the luteal phase were observed.

Endometrial Development

Echographic assessment of endometrial thickness was performed during the follicular phase in the baseline cycles and throughout all treatment and post-treatment cycles. The maximal thickness attained during each of the three periods of treatment with mifepristone was significantly lower than the maximum observed during the follicular phase of the baseline cycles (X ± SEM, n = 10, baseline: 11.9 ± 1.0; mifepristone: 9.2 ± 0.6; 10.1 ± 0.4; 8.2 ± 0.5 mm, $p < 0.05$, ANOVA). During MPA treatment periods, maximal values in the first two periods were lower than those observed during the luteal phase of the post-treatment cycles (X ± SEM, n = 10, posttreatment: 13.8 ± 1.0; MPA: 11.2 ± 0.9; 10.6 ± 0.6; 12.8 ± 0.9 mm, $p < 0.05$, ANOVA).

All endometrial samples showed either delayed or irregular development. The endometrium was classified as secretory according to Noyes criteria in 5 women, but in all of them maturation was retarded, from 3 to 6 days, when the first day of MPA was taken as day 15 of a normalized cycle. The other five women had irregular endometrial development, with mixed types of glands or asynchronous with the stroma, but none presented hyperplasia.

Post-treatment Cycles

Only 9 cycles were followed and 5 of these were ovulatory, one of them without a detectable LH midcycle peak. The other four cycles were anovulatory, three of them biphasic, associated with an unruptured follicle and the other one, monophasic.

Side Effects

Total compliance of pill dosage was recorded by all volunteers in their diaries. No untoward reactions were reported. Clinical chemistry tests done before, during and after treatment did not show any particular trend among the few values lying outside the normal range.

Discussion

These results show that the sequential treatment with 5 mg/day of mifepristone for the first 15 days of the menstrual cycle followed by 10 mg/day of MPA for the following 13 days, during three consecutive months, inhibited ovulation in 63% of the cycles. The efficacy for preventing ovulation increased from 50% to 80% throughout the three treatment months. The tested regimen was totally effective in five women, partially effective in three and totally ineffective in two women. This result bears no correla-

Table 3. Effect of RU486-MPA sequential regimen on the menstrual bleeding*

Cycle	Interval Between the Last MPA Pill to Onset of Bleeding** (days)	Duration of Bleeding/ Spotting (days)	Length of the Cycle (days)
Baseline		4.1 ± 0.3	26.4 ± 0.7
I	1.7 ± 0.6	3.4 ± 0.3	29.7 ± 0.6
II	2.6 ± 0.3	4.1 ± 0.3	28.9 ± 0.8
III	7.3 ± 2.5[a]	4.6 ± 0.9	32.7 ± 2.3[b]
Post-treatment		3.4 ± 0.2	27.7 ± 1.7

*Values are mean ± SEM (n = 10).
**Both days excluded
[a]Different from cycles I and II p = 0.019 (Wilcoxon).
[b]Different from baseline p = 0.018 (ANOVA).

Table 4. Effect of RU486-MPA sequential regimen on the length of biphasic cycles and its phases

	Baseline Cycle n = 10	Treatment Cycles 1st Cycle n = 5	2nd Cycle n = 6	3rd Cycle n = 3	Post-treatment Cycle
Total length of cycle	26.4 ± 0.7	30.4 ± 0.4[a]	29.0 ± 0.9	29.7 ± 0.3[a]	28.4 ± 2.0 n = 8
Follicular phase	13.4 ± 0.6	18.6 ± 0.4[a]	17.3 ± 0.7[a]	16.3 ± 0.7	15.0 ± 1.9 n = 7†
Luteal phase	13.0 ± 0.6	11.8 ± 0.5	11.7 ± 0.8	13.3 ± 0.3	14.1 ± 0.7 n = 7†

Data are X ± SEM.
[a]Different from the respective baseline cycles $p < 0.001$, paired t test.
†Excluded 1 cycle in which there was no LH peak.

tion with body weight or mifepristone blood levels of these subjects (not shown).

In the ovulatory cycles, follicles grew reaching 16–20 mm at the end of mifepristone administration (day 15 of the cycle) and a delayed follicular rupture, with respect to that observed in the baseline cycles, took place during the period of treatment with MPA. In a previous study, the daily treatment with mifepristone, 5 mg/day for 30 days, inhibited ovulation in the five treated subjects, and follicular diameters attained were always less than 13 mm on day 15 of treatment.[4] The reason for this discrepancy between the two studies is unknown to us but it could explain the unexpected high rate of ovulatory cycles observed here. It is also possible that, regardless of the leading follicle size at the onset of the MPA treatment, this progestin at the dose used, instead of inhibiting pituitary gonadotropin secretion, triggers it when the effect of the antiprogestin begins to wane. In other studies, in which the antiprogestin was followed by NET or MPA,[5–7] the LH peak and the rise of progesterone also occurred during the period of treatment with the progestin.

The effects of MPA treatment on the bleeding pattern and endometrial cyclicity were as expected. Regularity of bleeding episodes was higher in the first two treated cycles than in the third cycle of treatment, in which MPA administration was not followed by mifepristone.

Endometrial secretory changes were induced by the progestin in all cases. However, irregular development, reflected in mixed type of glands or delayed endometrial maturation was observed. Endometrial alterations observed 6 to 8 days after discontinuing mifepristone suggest a long-lasting effect not readily reverted by the progestin. No signs of hyperplasia were detected upon histologic examination. Instead, reduced endometrial thickness was observed both during mifepristone and MPA administration, even in those cycles with high estradiol levels. This is consistent with the reported antiproliferative effect of mifepristone.[14]

A high proportion of cycles with unruptured enlarged follicles was found among monophasic cycles in the present study (11 of 16). Enlargement always occurred during treatment with the progestin. In most cases, their size was reduced until vanishing during the next mifepristone treatment period but they persisted into the post-treatment cycle when MPA treatment was not followed by the antiprogestin. A similar course was followed by the high estradiol levels that were observed in four cycles during MPA treatment. In two of them, they decreased rapidly when mifepristone treatment was reinitiated but, in the other two cycles, in which mifepristone was not given after the MPA, the high E2 levels were maintained for several days. The number of observations is too small to definitely conclude that RU486 has the ability to decrease abruptly the abnormally elevated E2 levels; nevertheless, this is possible. As shown previously,[1,4,11] and confirmed in this study, mifepristone administration during the follicular phase interferes with the rise but does not decrease estradiol levels which are within the normal range. This effect of the antiprogestin has been shown to be temporally related with a delay in the growth of the dominant follicle.[11] In the case of enlarged follicles, excessive estrogen secretion appears to be shut off by the antiprogestin; however, the mechanism is yet unclear.

The effects of the sequential regimen on ovarian function were carried over into the post-treatment cycle in most subjects. Only three of them recovered their normal function during this time indicating that a period longer than one cycle is needed for total recovery.

Taken together, the current data indicate that mifepristone, at a lower dose than used in the previous schemes of sequential regimen, can suppress ovulation and that the administration of the progestin in

the second half of the cycle allows regular bleeding cyclicity. The endometrial alterations found may be accompanied by diminished endometrial receptivity and may contribute to the contraceptive potential of this regimen. However, until the validity of this concept is confirmed, the efficacy of the regimen tested for inhibiting ovulation appears too low for phase II clinical trials.

Acknowledgments

We thank Roussel Uclaf and Laboratorios Chile for their generous supply of mifepristone and medroxyprogesterone acetate, respectively, and the WHO Matched Reagents Programme, for the immunoassay reagents. We also wish to thank Mrs. A. Brandeis, Mrs. G. Bravo, Mrs. C. Llados and E. Nuñez for their technical assistance.

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OUTSTANDING CONTRIBUTION

Effects of long-term low-dose mifepristone on reproductive function in women

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Low-dose antiprogestin administration has been proposed as a new contraceptive modality to interference with endometrial receptivity without disturbing ovarian function. The effects of 1 mg/day mifepristone for 150 days on the menstrual cycle were assessed in 21 surgically sterilized women. The aim was to study each woman for one control cycle and during months 1, 3 and 5 of treatment. Ovulation, endometrial thickness, serum oestradiol and progesterone, urinary luteinizing hormone, endometrial morphology and cervical mucus were assessed. Luteal phase progesterone concentrations were observed in 36 of the 60 treated months assessed and less frequently as treatment progressed. The bleeding pattern was regular in most biphasic cycles, while prolonged interbleeding intervals or no bleeding were associated with monophasic cycles. Altered endometrial morphology was found in all cases irrespective of the occurrence of luteal activity. Increased endometrial thickness and dilated glands were observed in 25 and 34% respectively of the monophasic cycles. Mifepristone, 1 mg/day, interferes with endometrial development while allowing the occurrence of biphasic ovarian cycles and regular bleeding. However, it also prevents ovarian cyclicity in a high proportion of treated months, and this is associated with increased endometrial growth in some women, which may be of concern.

Key words: antiprogestin/endometrial contraception/mifepristone/ovarian cycle/women

Introduction

The effects of progesterone blockade by mifepristone administration during the menstrual cycle in women indicate that antiprogestins may be used to inhibit ovulation and/or to prevent implantation (for recent reviews see Van Look and von Hertzen, 1995; Spitz *et al.*, 1996). Preliminary trials have shown that daily mifepristone administration throughout one menstrual cycle inhibited ovulation and altered endometrial development when doses ≥2 mg/day were used (Ledger *et al.*, 1992; Croxatto *et al.*, 1993; Cameron *et al.*, 1996). A differential threshold for mifepristone effects on the ovary and the endometrium was observed when 1 mg/day was given (Croxatto *et al.*, 1993). With this dose, endometrial development was consistently altered and ovarian function was preserved in most cases (Batista *et al.*, 1992; Croxatto *et al.*, 1993). This differential threshold would theoretically allow for inhibition of endometrial development and function, thus preventing implantation, while ovarian function and bleeding cyclicity would be preserved. To explore this hypothesis, a two-centre trial was conducted to assess the effects of 1 mg/day mifepristone for 5 months on ovarian function, endometrial development and bleeding cyclicity. The recent publication of a similar study performed utilizing 0.5 or 0.1 mg/day mifepristone (Gemzell-Danielsson *et al.*, 1997) complements the findings reported here.

Materials and methods

The study was approved by the ethics committee at the Albert Szent-Györgyi Medical University (Szeged, Hungary) and at ICMER (Santiago, Chile). Each volunteer gave written informed consent before being enrolled in the study. A total of 23 healthy, surgically sterilized women volunteered for the study: 11 at Szeged, Hungary and 12 at Santiago, Chile. Mean age, height and weight were 34.6 years (range 25–39), 158.4 cm (range 150–168) and 59.8 kg (range 50–85) respectively. A medical and gynaecological examination, routine serum chemistry analysis and haematological investigation were performed at admission and at the end of the study.

One woman discontinued voluntarily during the control cycle. Therefore only 22 of the 23 women began treatment, and 19 of these completed the study. Each woman who completed the study participated for one control cycle and one 150 day treatment period. One woman contributed to the study with only 120 days and another with only 60 days of treatment; their data were included in the analysis. One woman was excluded from further analysis after an abnormal control cycle was detected. Altogether 101 months of treatment were accrued by 21 women. Treatment started on day 1 of the cycle, immediately after the control cycle. During treatment, the subjects received 1 mg/day mifepristone orally, between 6:00 and 10:00 h. The endpoint variables were assessed during the control cycle and months 1, 3 and 5 of treatment. All subjects were asked to keep daily records of pill intake time, bleeding and spotting episodes, complaints and other medications.

Ovarian function assessment

Ovarian function was evaluated according to the growth and rupture of the leading follicle and the concentration of oestradiol and

progesterone in the plasma. Follicular growth and the occurrence of ovulation were assessed by ultrasonography twice a week during the control cycle and three times a week during months 1, 3 and 5 of treatment using an Aloka SS D 620 (Tokyo, Japan) with a 5 mHz vaginal probe or a Hitachi EUB 450 (Tokyo, Japan) with a 7.5 mHz vaginal probe.

Blood samples, 10 ml each, were obtained on the same days as echographic monitoring. The concentrations of oestradiol and progesterone in the plasma and of luteinizing hormone (LH) in the urine were measured by a radioimmunoassay, according to the procedures and with the reagents supplied by the World Health Organization (WHO).

Assessment of endometrial development

Endometrial thickness was measured by ultrasound on the same days of follicular growth assessment. Measurements were performed in the sagittal plane, across the upper third, from one basal layer to the other. The thickness of the luminal fluid image, when present, was subtracted from the measurement (Scott, 1994). Endometrial biopsies were taken with a cannula (GynoSampler, GynoFarma) or a 5 mm Randall curette during the control cycle and during months 1, 3 and 5 of treatment. The study protocol prescribed that the biopsy should be taken: (i) on days 22–24 of the pretreatment cycle; and (ii) 8–10 days after an LH peak or on days 22–24 of an LH peak-free interval, whichever came first, during months 1, 3 and 5 of treatment. To time the endometrial sampling according to the LH peak, volunteers collected daily the first morning urine sample.

In the control cycles 21 endometrial biopsies were taken: 16 on days 7–10 after follicular rupture, and five on days 1, 4, 5, 11 and 12 after follicular rupture. During the first month of treatment 21 biopsies were taken: 15 on days 20–24 and six on days 25–28 of treatment. In the third month 18 biopsies were taken: 15 on days 83–86 and three on days 79–80. In one subject the biopsy was taken on day 92 of treatment, and in another no biopsy was taken. During the fifth month 16 biopsies were taken: 10 on days 143–147, four on days 134–142 and two on days 148–149 of treatment. In four subjects no biopsy was taken during this month. Part of each tissue sample was fixed in 10% buffered formalin and embedded in paraffin. Tissue sections were stained with haematoxylin–eosin to assess endometrial histology. The remaining tissue samples were processed for steroid receptor immunocytochemistry (results not reported here).

Endometrial dating was performed according to the criteria of Noyes *et al.* (1950) at each centre. Later on, all samples were reviewed by the pathologist at Santiago (H.D.C.). Samples that did not fit with Noyes's criteria were classified as follows: (i) secretory irregular: uneven glandular growth, with or without intraluminal secretion, and with varying degrees of stromal oedema, with rare slight-to-moderate predecidual reaction; (ii) secretory delayed: secretory pattern that does not correspond to the histological picture of the endometrium expected according to the post-LH peak interval; (iii) mixed: proliferative and secretory signs in different endometrial glands; or (iv) involuted: small glands exhausted of secretory material, lined with cuboidal or low columnar eosinophilic epithelium; the appearance of the stroma is variable, but almost always displays some degree of oedema.

Cervical mucus

Cervical mucus samples were taken two or three times a week before the ultrasound examination during the control cycle and months 1 and 5 of treatment in a subgroup of 11 women. Evaluation included the assessment of the amount, consistency, spinnbarkeit, ferning and cellularity, according to the procedure described by WHO (1987). The scale for each variable was from 0 to 3, allowing a maximum total score of 15.

Data analysis

In the analysis of the data the following end-points and definitions were used: (i) length of the cycle: cycle length was calculated counting from day 1 of menses until the day preceding the next menstrual-like bleeding, both inclusive (if it lasted >90 days it was considered amenorrhoea); (ii) retrospective timing of the endometrial biopsy: the day of the cycle in which each endometrial biopsy taken was related to day 1 of the luteal phase, unless it was taken in the follicular phase. The first day in which the follicular echo-image disappeared was designated day 1 of the luteal phase. Usually this day coincided with the day of the LH peak in urine. Therefore LH peak in urine, followed by at least a doubling of progesterone concentrations, was used in some instances as an alternative criterion when the first was not available, e.g. luteinized unruptured follicle; (iii) follicular rupture: abrupt disappearance or a reduction in size of at least 50% of the echo-image; (iv) ovulation: follicular rupture followed by plasma progesterone concentrations >12 nmol/l in at least two samples taken during the luteal phase; (v) enlarged follicle: follicle with a mean diameter >25 mm; luteinized unruptured follicle: persistent echo-image of a follicle, associated with increased plasma progesterone concentrations; biphasic cycle: cycle in which plasma progesterone concentrations were >12 nmol/l in at least two samples, otherwise it was monophasic; cycle with uncertain endocrine profile: menstrual cycle partially monitored in which it was not possible to assess the occurrence of a luteal phase, e.g. a prolonged follicular phase which ran through the 30 day assessment period.

Statistical analysis

The proportion of women who exhibited ovulation or biphasic cycles at each assessment period was analysed by logistic regression (Hosmer and Lemeshow, 1989). An analysis of variance was used to compare the length of the cycles. The paired *t*-test was used to compare the maximum plasma hormone concentrations (oestradiol and progesterone) and the number of days with a cervical mucus score ≥10 between periods with and without luteal activity. The proportion of cycles with and without luteal activity that had endometrial thickness ≥18 mm was compared using Fisher's exact probability test.

Results

Ovarian function

Control cycles were biphasic in 21 women and ovulatory in 20 women. The subject who did not ovulate had a luteinized unruptured follicle which was not considered to be a reason for exclusion from the analysis.

During treatment, 60 treated months were assessed. Of the 21 women, 14 ovulated at least once during treatment. Four women ovulated in each of the 3 months assessed, three women in two and seven women in only one of them. The other seven women were anovulatory at each assessment period. The distribution of ovulatory cycles throughout the months of treatment is shown in Figure 1. The proportion of ovulatory cycles was highest during month 1 and decreased progressively with treatment. Using a logistic regression analysis the statistical significance was borderline ($P = 0.06$). However the odds ratio (slope) between months 1 and 5 of treatment was 3.7, which was significant.

According to the hormonal pattern there were 36 biphasic

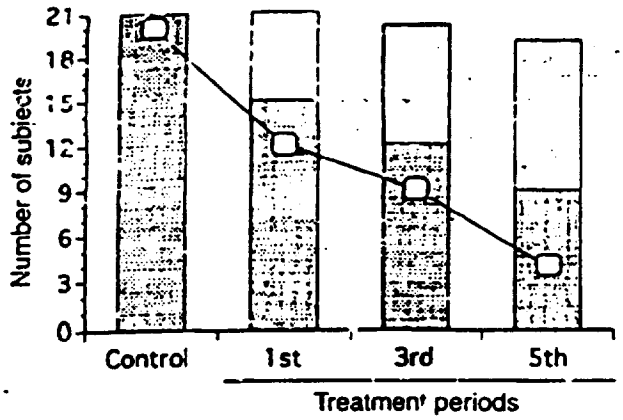

Figure 1. Ovarian function during treatment with 1 mg/day mifepristone for 5 months. Each bar represents the number of subjects with biphasic (shaded bars) and monophasic cycles (open bars) during the control cycle and during months 1, 3 and 5 of treatment. Open symbols represent the number of ovulatory cycles observed in each period.

and 24 monophasic periods. The proportion of biphasic cycles tended to decrease during treatment (Figure 1). Using a logistic regression analysis this tendency was not found to be statistically significant. Ovulation was confirmed echographically in 25 of the 36 biphasic periods. Another six of the biphasic periods corresponded to luteinized unruptured follicles. The five remaining biphasic periods had luteal activity but the critical part of the follicular phase fell outside the assessment period, therefore the occurrence of follicular rupture could be neither confirmed nor excluded. The length of the luteal phase and maximum progesterone concentrations in the ovulatory cycles and in those with unruptured follicles were not significantly different from those observed in the control cycles (data not shown).

An enlarged follicle was found in 13 of the 60 (22%) assessment periods [mean ± SE, 31.5 ± 1.2 mm in diameter (range 27–40)]. In 10 women it was an isolated finding, but in one woman an enlarged follicle was found at each assessment. Follicular enlargement was associated with high oestradiol concentrations (1500–2500 pmol/l) in the three instances in this subject and once in another case. Excluding these two subjects, maximal plasma oestrogen concentrations observed during the periods without luteal activity (mean ± SE, 528.0 ± 57.9 pmol/l) were lower than in those with luteal activity (748.0 ± 55.6 pmol/l) ($P < 0.001$).

Cycle length and bleeding pattern

In all, 21 women recorded their bleeding and spotting episodes during a total aggregate of 101 months of treatment. Their bleeding records show the occurrence of 77 interbleeding periods.

A regular bleeding pattern (range 22–38 days) was observed throughout treatment in nine of the 21 women, allowing the identification of five cycles in each of seven women, six cycles in another and two cycles in one woman who was treated for only 60 days (Figure 2A). In these women the first three treated cycles were slightly longer than the control cycle

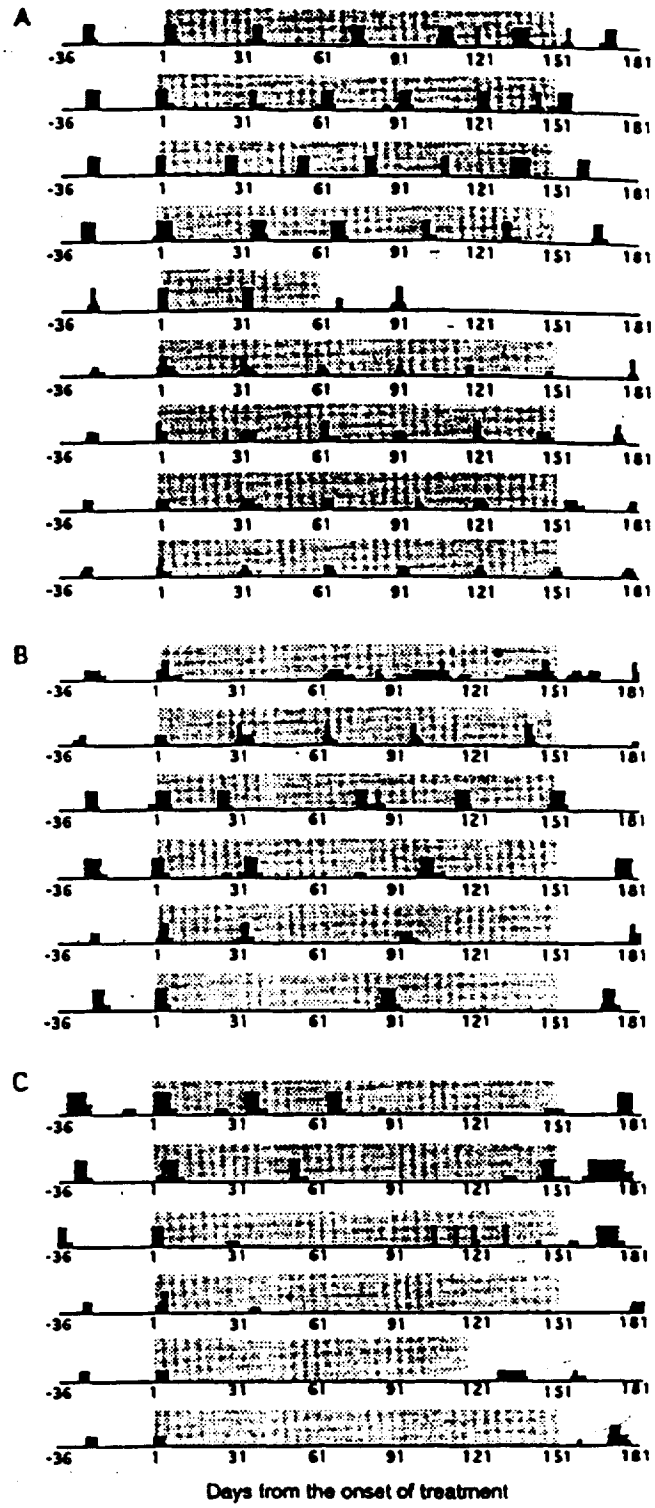

Figure 2. Bleeding pattern during 5 months of treatment with 1 mg/day mifepristone. (A) Subjects with a regular bleeding pattern during all treatment. (B) Subjects with an irregular bleeding pattern and (C) subjects with amenorrhoea. Black areas represent bleeding episodes. The height of the black bars indicates the daily amount of bleeding (small, spotting; medium, normal; large, heavy). Shaded areas represent the period of treatment.

[mean ± SE, 27.0 ± 0.6, 31.3 ± 1.0, 31.0 ± 1.2 and 30.9 ± 1.3 days ($P < 0.02$) for the control cycle and the first three treatment cycles respectively]. The length of the last two treated cycles (27.8 ± 0.9 and 29.9 ± 1.3 days) was not different from the control.

An irregular bleeding pattern, with cycle lengths ranging from 22 to 84 days, was found in six women (Figure 2B), and amenorrhoea during the entire treatment or a segment of it was observed in a further six women (Figure 2C).

Out of 42 cycles within the range of 22–38 days, 31 were biphasic, seven were monophasic and in four the endocrine profile was uncertain. In contrast, of the 12 cycles within the range of 38–90 days, four were biphasic (44, 62, 52 and 83 days), five were monophasic and three were uncertain. Amenorrhoea occurred in six women. In four women the period of amenorrhoea was monophasic, in one the endocrine profile was uncertain and in another, who had amenorrhoea for 129 days, there was a progesterone rise between days 25 and 37 (similar to that of a normal luteal phase but no bleeding was observed when progesterone concentrations fell).

Effects on endometrial development

In the control cycles 21 biopsies were taken, one of which was insufficient for an analysis. A secretory endometrium with a dating according to the LH peak was found in only 13 of the 20 samples. Another five samples corresponded to secretory irregular endometrium, associated in three cases with abnormal findings: one of them with dilated glands, another with tubal metaplasia and the third with epithelial glandular metaplasia. Signs of metaplasia were not observed during treatment in these women. Another two samples corresponded to involuted endometrium, one taken on day +11 of the luteal phase and the other during an insufficient luteal phase.

During treatment 56 biopsies were taken, three of which were insufficient for analysis. Secretory signs were observed in 39 biopsies, nine were proliferative and five were involuted. Among the 39 samples with secretory signs, only one taken during the first month of treatment, on day 9 after ovulation, had an endometrial dating in agreement with the timing of ovulation; all others corresponded to irregular, delayed or mixed endometrium.

In all, 20 of the samples exhibiting secretory signs were obtained during the follicular phase, and the remaining 19 were taken during the luteal phase. All samples showing proliferative endometrium were taken in the follicular phase, whereas involuted endometrium was found in both phases.

Among the samples taken during the luteal phase, 15 were removed on days 7–10 of the luteal phase, with progesterone concentrations within the range 20–75 nmol/l; these are listed individually in Table I.

Table II summarizes the results of the endometrial assessment performed during treatment in the subgroup of women who had normal endometrium during the control cycle. Only one of the 29 biopsies, not classified as proliferative, exhibited normal development. In the subgroup of women who had an abnormal endometrium during the control cycle, the alterations of the endometrium found in the control cycles did not worsen during treatment. On the other hand, the alterations found in samples taken during treatment in these subjects were not different from those found in the normal subgroup.

Dilated endometrial glands were observed in 18 of the 53 samples taken during treatment. Of these, 11 samples were taken during cycles longer than 39 days and the other seven during cycles within the normal length, five of which were ovulatory.

The range in value of maximal endometrial thickness observed in 21 control cycles was 6–16 mm. Values ranging from 18 to 28 mm were observed in nine of the 60 assessment periods during treatment. Maximal endometrial thickness was increased to ≥18 mm during treatment in six of the 19 subjects who completed the study: this was detected only in treatment months 1, 3 or 5 in three subjects, in months 1 and 5 in one subject and in months 3 and 5 in two subjects. Thus the longer the duration of treatment, the greater the chance of encountering increased endometrial thickness. Two instances of increased maximal endometrial thickness were observed during 36 treatment cycles with luteal activity. In contrast, maximal endometrial thickness was increased to ≥18 mm in seven of 24 cycles (29%) with no luteal activity ($P = 0.023$; Table III). This increased thickness was associated with amenorrhoea in one woman (28 mm). The women in four of the seven instances also had dilated endometrial glands. Maximal plasma oestradiol concentrations in these seven cycles were not increased and remained within the range 310–660 pmol/l. All increases in maximal endometrial thickness took place in women who had a normal endometrial morphology in their control cycle. Six of the seven increases in endometrial thickness to ≥20 mm were observed in women whose maximal thickness in the control cycle was in the upper end of the range (14–16 mm).

Effects on cervical mucus score

Treatment had no significant effect on cervical mucus score (CMS) except for an increase in the number of days with a CMS ≥10 in periods with no luteal activity compared with periods with luteal activity ($P = 0.03$). During the control cycle the 11 subjects sampled had luteal activity; their CMS were ≥10 for 0–2 days. During the first month of treatment the CMS was ≥10 for 2 and 5 days respectively in two subjects who had no luteal activity; the CMS was ≥10 for 0–3 days in the nine women who had biphasic cycles. During the fifth month of treatment, nine women were evaluated. The number of days with a CMS ≥10 was in the range 0–9 in those with no luteal activity ($n = 5$) and 1–3 in those with biphasic cycles ($n = 4$), with a median of 4.0 and 2.5 days respectively.

Side-effects

No untoward reactions were reported by the volunteers. Clinical chemistry, blood cell counts and urine analyses were performed at enrolment and at the end of the third and fifth treatment periods. The chemical analysis included measurement of serum glutamic oxalacetic and pyruvic transaminase, lactic dehydrogenase, alkaline phosphatase, bilirubin, total protein, cholesterol, uric acid, urea nitrogen, glucose, inorganic phosphate and calcium. All were within the normal range.

Table I. Endometrial morphology on days 7–10 of the luteal phase during continuous treatment with 1 mg/day mifepristone

Subject no.	Treatment month	Day of luteal phase	Endometrial morphology (dating)	Progesterone[a] (nmol/l)
15	1	+9	Secretory normal (day 22)	55.0
16	1	+7+8	Secretory irregular	53.5
17	1	+7	Secretory irregular	35.2
18	1	+8+9	Secretory delayed (day 17)	54.8
19	1	+8+9	Secretory delayed (day 17)	75.0
21	1	+8+9	Secretory delayed (day 17)	31.7
12	3	+9	Secretory irregular	30.6
15	3	+9	Mixed	36.7
16	3	+7	Secretory delayed (day 17)	56.5
19	3	+9	Secretory irregular	75.0
21	3	+8+10	Secretory delayed (day 17)	NM
9	5	+8	Secretory delayed (day 17)	19.6
15	5	+10	Secretory delayed (day 18)	67.6
19	5	+8+9	Secretory irregular	75.0
21	5	+8+10	Secretory delayed (day 17)	37.0

NM = not measured.
[a]Progesterone concentrations on the day of endometrial biopsy.

Table II. Endometrial morphology during continuous treatment with 1 mg/day mifepristone in subjects who had normal endometrium before treatment

	n[a]
Proliferative	6[b] (2)
Secretory normal	1
Secretory irregular or delayed	18 (5)
Mixed	7 (4)
Involuted	3 (2)

Values in parentheses are the number of samples that presented dilated glands.
[a]In all, 13 subjects contributed three biopsies each. The four missing biopsies correspond to insufficient sample (n = 1) and biopsies not taken in the third and fifth treatment month.
[b]Endometrial biopsies taken before the progesterone rise.

Table III. Maximal endometrial thickness according to luteal activity during continuous treatment with 1 mg/day mifepristone

Periods	Luteal activity[a] mm [mean ± SD (n)][b] [outlying values][c]	No luteal activity mm [mean ± SD (n)] [outlying values]
Control	11.7 ± 2.9 (21)	
Treatment		
Month 1	11.2 ± 2.3 (14) [22]	9.6 ± 1.9 (6)
Month 3	11.2 ± 2.7 (11) [19]	12.7 ± 1.8 (6) [20,22]
Month 5	12.3 ± 1.7 (9)	12.4 ± 4.1 (5) [18,20,24,27,28]

[a]Plasma progesterone concentrations >12 nmol/l in at least two consecutive samples taken 1 or 2 days apart.
[b]Outlying values >16 mm were excluded from the calculation of mean ± SD (n).
[c]Proportion of outlying values in periods with versus without luteal activity (P = 0.023).

Discussion

Mifepristone given continuously at a dose of 1 mg/day disrupted endometrial development in all subjects during treatment. Only one of the 53 samples exhibited a secretory endometrium in phase with the hormonal events, and this occurred in the first treatment cycle. However, 40% of the cycles were monophasic, and bleeding cyclicity was altered in 57% of cases—a much larger proportion than desirable. These results suggest that a lower dose might still affect endometrial maturation without altering ovarian sex hormone secretion. Recently Gemzell-Danielsson *et al.* (1997) reported that the continuous daily administration of 0.5 mg/day mifepristone for 3 months did not alter follicular growth, oestrone glucuronide, pregnanediol glucuronide, LH concentrations in urine or cycle length, and that all five subjects ovulated during treatment. At the same time endometrial development appeared to be slightly retarded and exhibited decreased binding of *Dolichos biflorus* agglutinin and decreased glycodelin expression. A dose of 0.1 mg/day had no significant effect on the endometrium. It remains to be seen to what extent the endometrial effects of 0.5 mg/day mifepristone exert a contraceptive effect.

The study of Gemzell-Danielsson *et al.* (1997) and our study do not resolve the question of the feasibility of endometrial contraception using a continuous low-dose antiprogestin but provide a fair basis to select a dose for a phase 2 clinical trial and also a basis for comparison between different antiprogestins in future studies. An alternative regimen for endometrial contraception, which appears to be particularly suitable for mifepristone in view of its long half-life in the circulation (Deraedt *et al.*, 1985; Kekkonen *et al.*, 1996), is the intermittent or once a week administration reported by Gemzell-Danielsson *et al.* (1996). The once-weekly administration of 5.0 or 2.5 mg delayed endometrial development and impaired secretory activity without inhibiting ovulation. The closest proof of the feasibility of endometrial contraception so far has been the single administration of 200 mg mifepristone in the early luteal phase (Gemzell-Danielsson *et al.*, 1993). When the contraceptive efficacy of this regimen was tested in 21 unprotected women in a total of 157 ovulatory cycles, only one clinical pregnancy occurred.

Another important issue concerned with the use of con-

tinuous low-dose antiprogestins for endometrial contraception is safety. It has been feared that the administration of antiprogestin for prolonged periods of time will expose the endometrium to continuous oestrogen action not antagonized by progesterone, and that this could be unsafe for women. In this study the only endometrial alterations found that relate to this concern were increased endometrial thickness and dilated endometrial glands. Six of the 19 subjects who completed the study, who had increased endometrial thickness up to ≥18 mm, represented the affected group. Although the numbers were too small to draw any conclusions, there was a tendency to encounter these effects more frequently in the later than in the earlier assessment periods. Similarly, the largest increases in endometrial thickness (24, 27 and 28 mm) were only observed in the fifth month of treatment. Another important feature was that thickening was significantly more likely to occur in assessment periods with no luteal activity. Because most of the increases were associated with neither amenorrhoea nor elevated oestradiol concentrations, they cannot be explained by stronger or longer oestrogenic stimulation. It is more likely that unopposed oestrogen stimulation acting on predisposed individuals is responsible for greater endometrial growth. This is difficult to prove, but the fact that subjects who had a thicker endometrium in the control cycle were more likely to have increased endometrial thickness during treatment suggests that this may be the case.

The finding of dilated glands in endometrial samples taken during treatment is in contrast to the significant decrease in the diameter of glands reported during the second month of treatment with 5 mg mifepristone administered once a week (Gemzell-Danielsson *et al.*, 1996) and during the third month of treatment with 0.5 mg/day mifepristone (Gemzell-Danielsson *et al.*, 1997). The different endometrial response observed in our study does not have an obvious explanation. In the studies of Gemzell-Danielsson *et al.* (1996, 1997), mean glandular diameter was calculated from morphometric assessment. In our study the labelling of a sample with dilated glands resulted from the visual assessment performed by the pathologist at low magnification; however, this methodological difference is unlikely to explain contradictory findings. In the study with 5 mg/week mifepristone, it is likely that partial suppression of oestradiol concentrations may have occurred. In addition, this dose may have a qualitatively different effect on the endometrium. In the study with 0.5 mg/day mifepristone only five women were studied, and this low number, combined with low incidence of the event, may have eliminated the chance of observing samples with dilated glands. The significance of this type of gland is not evident from these data because no signs of endometrial hyperplasia were observed.

The fact that secretory signs were observed in the endometrium of women with preserved ovarian endocrine function, despite the antiprogestin treatment, could be interpreted as incomplete blockade of progesterone action by the dose used. However, the same secretory signs were also observed in biopsies taken in periods with no luteal activity. Because it has been reported that mifepristone can have agonistic effects on the endometrium, in particular under conditions in which endogenous progesterone is present at low concentrations (Gravanis *et al.*, 1985), this may also be the case for these samples.

The results of this study confirm that endometrial maturation can be affected by the daily administration of low doses of mifepristone without necessarily altering ovarian sex hormone secretion. Whether or not those endometrial alterations will be sufficient to prevent implantation remains to be established.

A tendency for progressive effects over 5 months of treatment was detected at both the ovarian and endometrial level. Its significance is unclear, but it is advisable that this type of phase I study is conducted over extended periods of continuous drug exposure.

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OUTSTANDING CONTRIBUTION

Contraceptive potential of a mifepristone–nomegestrol acetate sequential regimen in women

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The effectiveness of a sequential regimen consisting of mifepristone, 10 mg/day for 15 days, followed by nomegestrol acetate (NOMA), 5 mg/day for the next 13 days, for inhibiting ovulation and maintaining regular bleeding cycles was assessed in 10 surgically sterilized volunteers who were followed for one pretreatment and three treated cycles. Hormonal determinations in blood and urine, ovarian ultrasonography, bleeding records in all cycles and an endometrial biopsy taken on day 22–25 of the third treatment cycle were used to monitor the effects of treatment. During treatment, 24 monophasic (no sustained progesterone rise above 12 nmol/l) and six biphasic cycles were recorded. Nine follicular ruptures were detected echographically in these 30 treated cycles, five of which occurred in monophasic cycles. All follicular ruptures occurred on days 1–7 of NOMA treatment. Echographic and endocrine features of ovulatory cycles were both present in only four treated cycles (13.3%). Development of a secretory endometrium was achieved in all cases, but it was always irregular. Regular withdrawal bleeding occurred in all subjects and no adverse reactions were recorded. The ovarian and endometrial effects of this regimen justify testing its contraceptive effectiveness in phase 2 clinical trials.

Key words: contraception/mifepristone/nomegestrol acetate/ovulation inhibition/women

Introduction

An antiprogestin–progestin sequential regimen has been proposed as an oral contraceptive method devoid of exogenous oestrogen and able to inhibit ovulation while maintaining regular bleeding cycles. In this regimen the antiprogestin is given for 15 days and the progestin for the next 13 days. This treatment cycle is repeated continuously without pill-free intervals.

The concept of this method arose from the demonstration that mifepristone, given during the follicular phase, arrests further follicular development and postpones ovulation (Liu *et al.*, 1987; Luukkainen *et al.*, 1988; Ledger *et al.*, 1992; Croxatto *et al.*, 1993, 1995). Thus, under mifepristone administration, ovulation is prevented but sufficient endogenous oestradiol is produced to stimulate endometrial growth. The progestin given following the antiprogestin course should have three effects: (i) prevent ovulation by a negative feedback on gonadotrophin secretion; (ii) antagonize temporarily the endogenous oestrogens; and (iii) transform the oestrogen-primed endometrium into a progestational endometrium, with consequent bleeding upon withdrawal of the progestin. The reinstatement of the antiprogestin, on the day after the last progestin pill is taken, should reinforce the effect of the progestin withdrawal upon the endometrium, ensuring the onset of bleeding, at the same time that it would prevent follicular escape.

Previous phase 1 studies, using mifepristone, 5 mg per day, as the antiprogestin, and norethisterone (Kekkonen *et al.*, 1990, 1993) or medroxyprogesterone acetate (Kekkonen *et al.*, 1993, 1995; Croxatto *et al.*, 1996) as the progestin, have shown that this regimen inhibits ovulation but not at sufficiently high rates to achieve an acceptable contraceptive efficacy if it were used by unprotected women. A luteinizing hormone (LH) rise, follicular rupture or the rise of progesterone occurred in some treatment cycles, always during the administration of the progestin. Contrary to expectation, in some instances, the progestin appears to trigger a pituitary gonadotrophin surge when the effect of the antiprogestin wanes (Kekkonen *et al.*, 1993, 1995; Croxatto *et al.*, 1996). This surge fails to produce a full ovulatory response in some cases, presumably in those in whom the follicle has not reached maturity.

Alterations in the bleeding pattern are associated with several highly effective contraceptive methods and they reduce their acceptability. The bleeding pattern observed with this regimen has proved to be quite regular in all cases and this has encouraged attempts to improve the rate of ovulation suppression.

In the present study, we investigated the efficacy of mifepristone, 10 mg per day for 15 days, to ensure arrest of follicular growth, followed by the progestin nomegestrol acetate (NOMA) for 13 days. At the dose of 5 mg per day, NOMA has been reported to have potent gonadotrophin-suppressing activity and to inhibit ovulation (Bazin *et al.*, 1987; Couzinet *et al.*, 1996). Because of the carry-over effect of this regimen into the next cycle, this phase 1 study encompassed three successive treatment cycles.

Materials and methods

A total of 10 healthy sterilized women, regularly cycling, mean age 36 years (range 30–40) and mean weight 59 kg (range 45.5–67.0) volunteered for the study. Subjects were admitted after giving informed consent. The study was approved by the Ethics Committee of ICMER and by the Eastern Virginia Medical School Institutional Review Board.

This was an open, non-randomized, phase 1 clinical study, in which each volunteer was her own control. Each subject was studied for one baseline cycle, three consecutive 28-day periods of treatment and one post-treatment cycle. During each period of treatment, each volunteer received mifepristone (RU486; Roussel-Uclaf, Romainville, France), 10 mg/day, on days 1 to 15 of the cycle and nomegestrol acetate, NOMA (Lutenyl; Laboratorios Silesia S.A., Santiago, Chile) 5 mg/day, on days 16 to 28 of the cycle. The entire sequence was reinitiated on the day following the last NOMA pill, regardless of the occurrence of menses.

Each subject recorded pill ingestion time, bleeding, spotting, any symptoms, concurrent illness and other drug intake. Haematology and serum chemistry (serum glutamic oxalacetic and pyruvic transaminase, lactic dehydrogenase, alkaline phosphatase, bilirubin, total protein, cholesterol, uric acid, urea nitrogen, glucose, inorganic phosphate and calcium) were assessed at admission, at the end of treatment and after the post-treatment cycle. Blood samples were taken twice a week throughout the study from each volunteer to determine oestradiol and progesterone concentrations. A first morning urine sample was collected daily for measuring LH concentration. Ovarian and uterine echography, using an Aloka SS D 620 ultrasound system, with a 5-MHz vaginal probe, were performed twice a week throughout the study, to assess follicular growth and endometrial thickness. An endometrial biopsy was taken in the third cycle of treatment, on day 7 to 10 of NOMA intake, to assess endometrial histology according to the criteria of Noyes *et al.* (1950) or Maqueo (1980).

Hormones in plasma and LH in urine were measured according to the procedures and with the reagents supplied by the World Health Organization. The lower limits of sensitivity for oestradiol, progesterone and LH assays were 100 pmol/l, 1.9 nmol/l and 1.6 IU/l respectively. The ranges of the intra-assay coefficients of variation of low, medium and high pool, for oestradiol, progesterone and LH were 6–7%, 5–10% and 4–6% respectively. The ranges of the inter-assay coefficients of variation were 12–21%, 12–15% and 8–16% for oestradiol, progesterone and LH respectively. Creatinine concentration in urine samples, final dilution 1:100, was determined by colorimetric assay, using creatinine (Sigma Chemical Co. St. Louis, MO, USA) as standard. Colour was developed using NaOH and picric acid and optical density was read, after 30 min, at 490 nm in a Microplate Autoreader (Boots-Celltech, Diagnostics Limited, Slough, UK). The inter-assay coefficient of variation of the low, medium and high standard concentration were 5%, 5.6% and 3.2% respectively.

Data analysis

The following working definitions were used in the analysis of the data:

Length of the cycle and of the phases: cycle length was calculated counting from the first day of menses until the day preceding the next menstrual-like bleeding, both inclusive. The day of maximum LH rise in urine, followed by at least a doubling of progesterone concentrations, or the first day in which the follicular echo-image disappeared, was designated day 1 of the luteal phase.

Follicular rupture: abrupt disappearance or a reduction in size of at least 50% of the echo-image.

Ovulation: follicular rupture followed by plasma progesterone concentration over 12 nmol/l, in at least two samples taken during the luteal phase.

Table I. Effect of mifepristone–nomegestrol acetate sequential regimen on ovarian function

Cycle	*n*	Highest oestradiol concentration (pmol/l) (mean ± SE)	Highest progesterone concentration (nmol/l) (mean ± SE)
Baseline	10		
Ovulatory*	10	711 ± 74	41.3 ± 3.9
Treatment	30		
Biphasic	6		23.6 ± 3.3[d]
Ovulatory	4	561 ± 70[a]	22.0 ± 4.9
LUF**	2	401 ± 141	26.8 ± 1.1
Monophasic	24	369 ± 31[b]	
Follicular rupture	5	416 ± 74[c]	15.3 ± 2.9***
No follicular rupture	19	357 ± 34[b]	5.8 ± 1.1

* All biphasic.
**Luteinized unruptured follicle.
***In each of these five cycles, progesterone level was above 12 nmol/l only in a single sample.
[a]Not significantly different from baseline cycle.
[b]Significantly different from ovulatory treated cycle. Analysis of variance (ANOVA), $P < 0.025$.
[c]Not significantly different from ovulatory treated cycle.
[d]Significantly different from baseline cycle; ANOVA, $P = 0.0072$.

Enlarged follicle: follicle with a mean diameter >25 mm.

Luteinized unruptured follicle: persistent echo-image of the dominant follicle, associated with increased plasma progesterone concentrations.

Biphasic cycle: cycle in which plasma progesterone concentrations rose over 12 nmol/l, in at least two samples, otherwise it was monophasic.

Differences in the length of the cycles, the highest plasma hormone concentrations (oestradiol and progesterone) and maximal endometrial thicknesses, between baseline and treatment cycles and within treatment, were compared using analysis of variance.

Results

Ovarian function

All baseline cycles were ovulatory (Table I, Figures 1 and 2). The highest plasma oestradiol concentrations were in the range 399–1171 pmol/l (mean ± SE: 691 ± 250 pmol/l), the luteal phase was 13.2 days on average (range: 10–15), and the highest progesterone concentrations were in the range 32–75 nmol/l (41.3 ± 3.9). The largest follicular diameters recorded in basal cycles prior to follicular rupture were in the range 15–21 mm (18.4 ± 0.7).

Throughout treatment, nine follicular ruptures were detected in five of the 10 participants, twice in each of four women and once in another. Three of these five women presented ovulatory cycles, one ovulated twice and two ovulated once each (Figure 1). The other five of these nine follicular ruptures were not followed by a luteal phase (see below) and were detected in three women.

The frequency of ovulatory cycles during treatment was greatly reduced (Figure 2). Follicular rupture was inhibited in 21 of the 30 cycles (70%). However, five of the nine follicular ruptures did not meet the endocrine criteria for ovulation, therefore 26 of the 30 cycles (86.6%) were considered anovulatory. Only six cycles were biphasic and four of these were

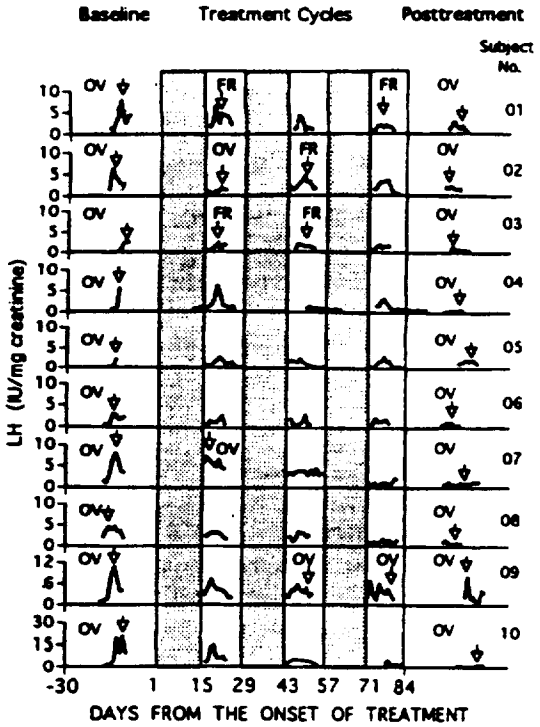

Figure 1. Effect of mifepristone–nomegestrol acetate sequential regimen on urinary luteinizing hormone (LH) concentrations and follicular rupture. Shaded and open lanes represent the periods of treatment with mifepristone, 10 mg/day for 15 days, and nomegestrol acetate, 5 mg/day for 13 days, respectively. The solid line shows LH concentrations, and arrows indicate the time when abrupt disappearance or a reduction in size of at least 50% of the echo-image of the leading follicle was observed. OV = follicular rupture followed by sustained rise in progesterone concentrations; FR = follicular rupture not followed by sustained rise in progesterone concentrations.

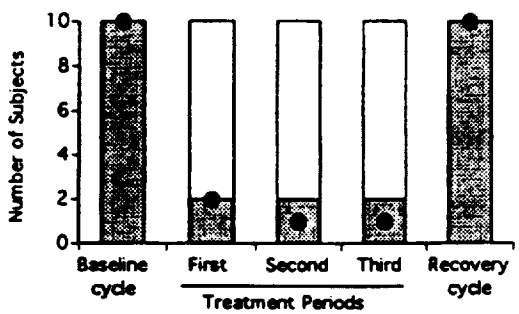

Figure 2. Ovarian function during mifepristone–nomegestrol acetate sequential regimen, as in Figure 1. Each bar represents the number of subjects with biphasic (shaded bars) and monophasic cycles (open bars) during one baseline, the three treatment and one post-treatment cycles. Closed symbols represent the number of ovulatory cycles observed at each period.

associated with echographic and endocrine features of ovulatory cycles. In the two remaining biphasic cycles, a luteinized unruptured follicle was observed. Follicular rupture was observed also in five of 24 monophasic cycles. All follicular ruptures occurred on days 1–7 of NOMA treatment. The largest follicular diameter recorded on days 13–15 of mifepristone was in the range 4–14 mm in 21 cycles and 15–20 mm in the other eight cycles. In one cycle, a follicle of 18 mm from

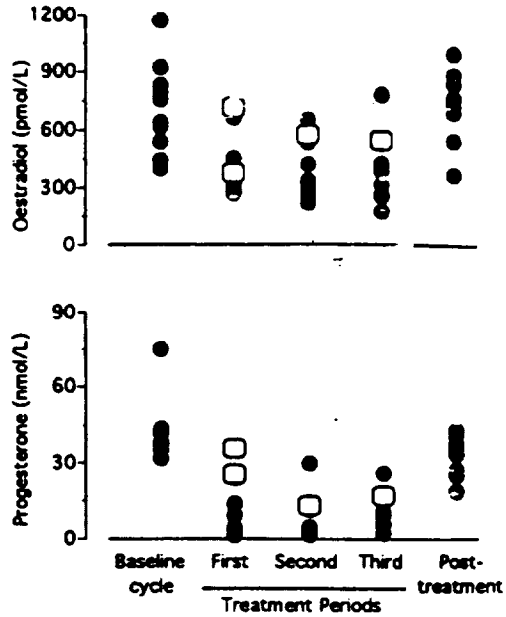

Figure 3. Highest plasma oestradiol and progesterone concentrations during mifepristone–nomegestrol acetate sequential regimen, as in Figure 1. Open symbols correspond to highest concentrations detected in ovulatory treated cycles.

the preceding cycle persisted growing during mifepristone treatment, attaining 25 mm, and became enlarged during the next progestin period, attaining 29 mm. In the nine cycles in which follicular rupture was detected, the largest follicular diameters observed on days 13–15 of mifepristone were in the range 10–20 mm and they reached 15–25 mm prior to follicular rupture. Maximal follicular diameters, on days 13–15 of mifepristone, in cycles in which no follicular rupture was detected were in the range 4–17 mm, excluding the enlarged follicle, with a median of 8.6 mm. Three enlarged follicles, with maximal diameters of 26, 28 and 29 mm were observed during treatment; all of them grew over 25 mm after the onset of NOMA intake and disappeared spontaneously at the time of the next menses.

A rise in LH concentrations was detected in urine, in 23 cycles during treatment. The magnitude and/or the sharpness of the surge was lower than in the baseline, in most cases. Follicular rupture occurred on the same day of the highest value only in three cycles, and two of them were considered ovulatory according to previously described criteria. In the other six cycles, the LH rise preceded follicular rupture by several days. In contrast, a clear LH peak was observed in two monophasic cycles (Figure 1). Creatinine concentrations in urine during treatment were not different from those in the baseline cycles.

Progesterone concentrations in biphasic cycles (n = 6) were significantly decreased (P = 0.0072) in comparison with those observed in the control cycles (Table I). The highest progesterone concentrations (Figure 3) observed in each of the four ovulatory cycles (22.0 ± 4.9 nmol/l, range: 12.7–34.8) were also significantly below (P = 0.04) the range observed during their corresponding control cycles (37.4 ± 1.5 nmol/l, range: 35.3–41.8).

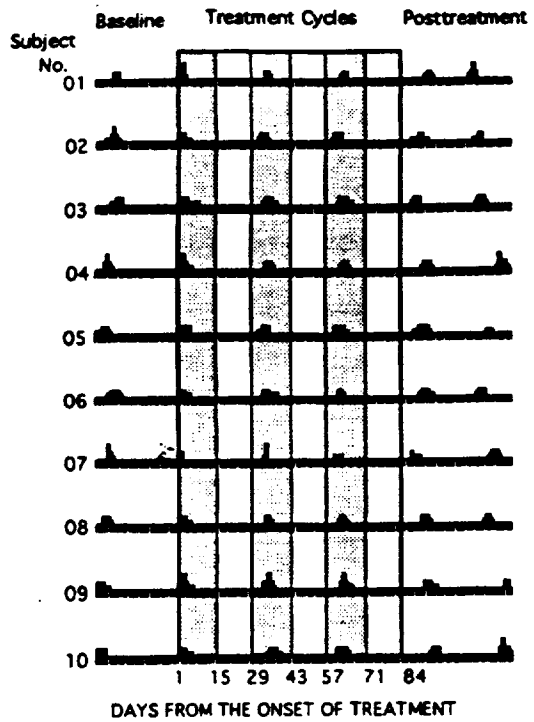

Figure 4. Bleeding pattern before, during and after three treatment cycles with mifepristone–nomegestrol acetate sequential regimen. Black areas represent bleeding episodes. The height of the black bars indicates daily amount of bleeding (low: spotting; medium: normal; and high: heavy). Shaded and open lanes as in Figure 1.

The highest oestradiol concentrations (Figure 3) in ovulatory treated cycles (561 ± 70 pmol/l, range: 383–723) were not different from those in their respective baseline cycles (685 ± 124 pmol/l, range: 440–831) but were significantly higher ($P < 0.025$) than in monophasic cycles (369 ± 31 pmol/l, range: 177–781). Highest oestradiol concentrations below 440 pmol/l were recorded in three women in the three treated cycles, in two women in two cycles and in another two women in a single cycle. In one cycle, oestradiol concentrations increased at the end of the NOMA period, remaining high during the 15 days of mifepristone intake (700–800 pmol/l), decreasing within the first 3 days of the next NOMA period. These oestradiol concentrations accompanied the growth of one enlarged follicle that attained a maximal diameter of 29 mm.

Cycle length and bleeding pattern

The first treated cycle (30–33 days in range), but not the second one (26–29 days), was longer than the baseline (24–30 days) due to a prolonged follicular phase ($P = 0.0001$). The third treated cycle (27–34 days) and its luteal phase were also longer than the baseline, probably due to the lack of an ensuing antiprogestin treatment period ($P = 0.0001$). The post-treatment cycle (16–30 days), in most cases, was shorter than the baseline ($P = 0.0237$).

The bleeding pattern during treatment was regular (Figure 4). Bleeding started 4, 4 and 6.5 days (median) after the first, second and third progestin treatment period respectively. As shown in Figure 4, the duration of bleeding episodes during treatment cycles (mean ± SD: 4.8 ± 1.6 days, range: 2–9) was not different from that of the baseline (5.3 ± 1.9 days, range: 2–9). Total absence of breakthrough bleeding or spotting was observed.

Table II. Effect of mifepristone–nomegestrol acetate sequential regimen on maximal endometrial thickness

Cycle	*n*	Thickness (mm)	
		Mean ± SE	Range
Baseline	10	14.1 ± 0.5	12–17
Treatment			
First	10	10.3 ± 0.7[a]	8–15
Second	10	9.9 ± 0.5[a]	7–12
Third	10	10.1 ± 0.3[a]	9–11
Biphasic	6	11.2 ± 0.8[a]	10–15
Monophasic	24	9.8 ± 0.3[a,b]	7–12
Post-treatment	10	11.3 ± 0.6[a]	9–15

[a]Significantly different from baseline cycle; analysis of variance, $P = 0.0001$.
[b]Not significantly different from biphasic cycle.

Endometrial morphology

The maximal endometrial thicknesses attained during the treatment cycles were lower than those observed during the baseline cycles, independent of the monophasic or biphasic profiles (Table II). Endometrial biopsies taken on days 7–10 of the third progestin treatment period showed disturbed development in all cases. All samples presented secretory signs but with a heterogeneous development of the glands, coexisting straight glands with stratified epithelium and coiled glands lined by high or low cylindrical epithelial cells, with secretion in vacuoles of basal and/or apical localization, and/or in the lumen. This glandular development was accompanied by a dense stroma, with infrequent oedematous areas in most cases, in which no vascular development or signs of focal predecidual reaction were observed.

Recovery cycles

All cycles were biphasic and associated with echographic features of the ovulatory cycle. These cycles were shorter than the baseline due in some cases to a shortening of the follicular phase and in others of the luteal phase. The maximal endometrial thicknesses in these cycles, although higher than in treatment cycles, were still below the growth attained in baseline cycles.

Side-effects

No untoward reactions or side-effects were recorded, and laboratory tests were within the normal range at the end of treatment.

Discussion

The sequential treatment tested affected ovarian function and endometrial development in all women in all cycles tested, albeit to a different degree, which ranged from total suppression of both follicular rupture and luteinization, or either one, to partial suppression of luteal-phase progesterone concentrations.

Some monophasic cycles were also associated with lower oestradiol peak concentrations. Except for the occurrence of follicular rupture in monophasic cycles, these polymorphic changes in ovarian function are to be expected from previously described effects of the antiprogestin on the pituitary–gonadal axis (van Uem *et al.*, 1989; Croxatto *et al.*, 1995). Follicular growth was partially inhibited in the majority of cycles during mifepristone treatment, and ovulation was less likely to occur in cycles exhibiting a stronger inhibition. The mechanism of this antifolliculotrophic effect of mifepristone remains to be disclosed, although the results of two recent studies, in which doses of 10 mg/day (Kazem *et al.*, 1996) or 50 mg/day (Messinis *et al.*, 1997) were used, suggest it is more likely a direct effect on the ovary rather than a disturbance of gonadotrophin secretion.

The absence of a clear pre-ovulatory LH peak in urine suggests either that oestradiol concentrations did not reach the critical level to exert a positive feedback or that a central effect of treatment interfered with the positive feedback of oestradiol on LH secretion (Baird *et al.*, 1995) The design of the study does not enable us to conclude whether this effect is due to antiprogestin, progestin or their interaction. Whatever the case, this effect persisted in the post-treatment cycle, since an LH surge, similar to that seen during pretreatment, was observed in only one case.

Maximal endometrial thickness was decreased during treatment. Even in the four ovulatory cycles with oestradiol peaks as high as in basal cycles, the thickness values found were below the range of basal cycles. It is most likely that this reflects the antiproliferative effect of mifepristone (van Uem *et al.*, 1989; Cameron *et al.*, 1996; Neulen *et al.*, 1996). On the other hand, 10 of the 24 monophasic cycles had maximal endometrial thickness values below 10 mm, whereas all six biphasic cycles had 10 mm or more. This difference did not reach statistical significance, probably due to the low number of biphasic cycles. Nevertheless, it suggests that the lower oestradiol concentrations encountered in monophasic cycles also contributed to a decreased endometrial proliferation.

The morphology of the endometrium, exposed to the exogenous progestin for 6 to 9 days, showed discrepant development among neighbouring glands as well as between the glands and stroma. The heterogeneous development of the glands was accompanied by scarce stromal development, in which vascular development and focal predecidual reaction failed to develop. This irregular development of the endometrium differs from that described for combined and sequential contraceptive pills (Dallenbach-Helweg, 1980; Maqueo, 1980). The changes in the stroma reflect a low to mild oestrogenic stimulus, followed by a sluggish response to the progestin. Glandular development was neither hyperplastic nor atrophic, as seen with the sequential and high-dose combination pills. The irregular development of glands suggests an uneven effect of the antiprogestin and/or the progestin on their target cells. Nevertheless, the most advanced development of glands was always far ahead of the stroma and behind that required for the synchrony with the embryo.

The occurrence of withdrawal bleeding, 2 to 5 days after reinitiating the antiprogestin pill intake, was highly predictable. The lengths of bleeding episodes were not different from those recorded before treatment. Breakthrough bleeding, intermenstrual spotting or amenorrhoea were not seen.

An acceptable contraceptive efficacy can be expected from the observed effects of this regimen on ovarian function and endometrial development. From the data, 13% of ovulatory cycles are to be expected with this regimen. Since follicular rupture occurs 7 to 13 days before restarting the antiprogestin intake, and implantation occurs 7 to 9 days after follicular rupture, if implantation should take place, it would be as early as 4–6 days before the next antiprogestin intake and as late as 1–3 days during progestin intake. In addition, endometrial stroma is delayed and the development of glands is highly irregular, therefore, it is unlikely that the endometrium would be receptive. Cervical mucus, which was not evaluated in this study, may be also affected by this treatment. Since follicular rupture takes place during the period of treatment with the progestin, it is likely that, in most instances, cervical mucus will be hostile to spermatozoa before and at the time of ovulation (Chretien *et al.*, 1991).

No serious adverse side-effects have been reported by women using the antiprogestin–progestin sequential regimen during this or previous studies (Kekkonen *et al.*, 1990, 1993, 1995; Croxatto *et al.*, 1996). Peak oestrogen concentrations in monophasic cycles were lower than in baseline cycles, however, enough oestrogen was secreted to stimulate endometrial growth, to prevent breakthrough bleeding and to render the endometrium responsive to the progestin and to its withdrawal. Ovarian cysts, reported for the continuous 10 mg mifepristone regimen (Croxatto *et al.*, 1993), were not seen with this regimen, and the few enlarged follicles observed disappeared at the time of next menses.

Based upon the results of this study and previous clinical experience with these steroids, the present mifepristone–nomegestrol acetate sequential regimen is expected to offer high contraceptive efficacy, excellent bleeding control and to be safe and relatively free of side-effects. It affords a definite advantage for women who cannot use the combined pill and for those who have low tolerance to bleeding disturbances.

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The effect of a single dose of mifepristone (RU486) on the fine structure of the human endometrium during the early luteal phase

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This study examined the fine structure of the human endometrial glandular epithelium after the administration of a single dose of RU486 (mifepristone), given in the early luteal phase. The drug was administered on days 2, 3, 5 and 6 after the luteinizing hormone peak (LH+0). Biopsies were performed on days LH+5, 6, 8 and 9. These were compared with control biopsies taken on corresponding days. Qualitatively, the main cytological effect of the RU486 was on the secretory apparatus and on the polarity of the cell. The formation of the nuclear channel system was also affected by the drug. A two-way analysis of variance on cell and nuclear volume data revealed no significant effect of day of biopsy, condition or interaction. Mitochondrial volume and secretory apparatus volume data revealed a significant effect of day of biopsy and interaction term; mitochondrial volume at LH+5 was 95.9 ± 25.3 μm³ (mean ± SD) for control and 57.7 ± 31.9 μm³ for RU486-treated epithelium. The volume of the secretory apparatus in the treated group was smaller on days LH+5 and 6 (14.6 ± 4.2 μm³, 6.41 ± μm³) when compared to day-matched control biopsies (35.9 ± 10.5 μm³, 41.7 ± 26.4 μm³). RU486 administration in the early luteal phase disrupted the secretory activity of the cells. These findings provide an insight into the cellular mechanisms of progesterone receptor blockade in the peri-implantation period.

Key words: electron microscopy/endometrial glands/mifepristone/morphometry/RU486

Introduction

RU486 (Roussel-Uclaf, Paris, France; also known as mifepristone) is a synthetic steroid which blocks the biological effects of progesterone at the receptor level (Baulieu, 1994). The most significant effects of progesterone are on the endometrium in the early luteal phase, where the hormone is necessary to allow implantation of the blastocyst and to support its development through early pregnancy. By abolishing these effects, RU486 is believed to prevent implantation, if given early enough in the luteal phase (Glasier *et al.*, 1992; Gemzell-Danielsson *et al.*, 1993), and to induce abortion if given after successful implantation has taken place (Hermann *et al.*, 1985; Schaison *et al.*, 1985; Shoupe *et al.*, 1990; Van Look and von Hertzen, 1995). The effect of RU486 on the endometrium depends upon timing of treatment and dose given (Gemzell-Danielsson *et al.*, 1996).

We have previously examined the precisely timed sequence of changes in the structure of the human endometrium through the first half of the luteal phase, in endometrial biopsies accurately dated from the surge in luteinizing hormone (LH): the subjects were carefully selected, normal women of known fertility (Li *et al.*, 1987, 1988a; Dockery *et al.*, 1988a,b, 1990).

Against this background of precisely dated reference data, we have reported (Li *et al.*, 1988b) the effects of a single dose of RU486 on the structure of the endometrium using light microscopy. RU486 inhibited secretory activity, accelerated degenerative changes, affected the stromal blood vessels, increased stromal, though not epithelial, mitotic rate and had no obvious effect on the predecidual reaction. In a subsequent study, Graham *et al.* (1991) reported that a single dose of RU486 given on LH+2 (where LH+0 was the LH peak) prevented the appearance of a luteal-specific secretory glycan, whereas administration of the drug on LH+5 did not prevent its expression. These studies, however, gave no insight as to the fate of the secretory triad (Cornillie *et al.*, 1985; Dockery and Rogers, 1989) of cellular events in the glandular epithelium.

Thus the aim of the present study was to redress these shortcomings by examining the effect of the administration of a single dose of RU486 given in the early luteal phase of the menstrual cycle on the fine structure of the human endometrial glandular epithelium.

Materials and methods

All women participating in this study were healthy volunteers who presented either for tubal sterilization or for the reversal of tubal sterilization. Women were only accepted into the study if they met the following criteria: (i) they were fertile, (ii) they had regular, normal menses with cycles of 25–35 days, (iii) they were aged 18–40 years, (iv) they had no demonstrable uterine pathology, (v) they had not used an intrauterine contraceptive device nor an oral contraceptive and had not received any hormone therapy in the previous 2 months. In addition, women recruited for the study had a baseline endometrial biopsy (biopsy 1) performed just before the administration of RU486 to ensure that they had normal endometrium; any individual whose biopsy was found to be abnormal was excluded from the study. For full details of these women, see Li *et al.* (1988b).

Ethical considerations

This study was approved by the Southern Hospitals Ethics Committee of Sheffield, UK. All women taking part in the study had explained to them the nature and purpose of the study, the procedures, the

Table I. Experimental design of the study

Day of biopsy	Number of women used in each group	
	Control	RU486
LH+5	5	4
LH+6	4	4
LH+8	4	4
LH+9/10	4	5
Total	17	17

LH = luteinizing hormone; LH+0 = luteinizing hormone peak.

possible side effects of RU486, and their right to withdraw from the study at any time. Each volunteer gave informed written consent before participating in the study.

Chronological dating

Daily LH assays were performed on blood or morning urine samples from all subjects, starting on day 9 of the menstrual cycle (Li *et al.*, 1987). The LH peak was identified from these assays, and all subsequent procedures were dated from it: LH+0 was the day of the LH peak.

Administration of RU486

A single dose of RU486 was given in the first half of the luteal phase between days LH+2 and LH+6. A variable dose of 5–200 mg was given to the women; an up-and-down design (Montecarlo stimulation) was used to determine the doses (See Li *et al.*, 1988b).

Endometrial biopsies

Full details are given by Li *et al.* (1988b). Baseline endometrial biopsies were taken immediately prior to RU486 administration.

Experimental biopsies

A second biopsy was systematically taken from each subject 3 days after administration of RU486. This was obtained from a site further from the first biopsy, so as to minimize any impact of the first biopsy on the morphological features studied. The first biopsy helped to ascertain that endometrial development prior to RU486 administration had been normal; consequently, it was possible to eliminate recruitment of patients with pre-existing abnormal biopsies. We had previously performed a pilot study (Li, 1988) and shown that the biopsy taken 3 days previously did not significantly alter the structural development of the endometrium at the light microscopic level according to the criteria of Noyes *et al.* (1950), although one patient showed an increased infiltration of lymphocytes. It is uncertain whether or not this would affect the ultrastructure of the glands. On the basis of the above two arguments, we felt justified in assuming that the first biopsy did not significantly alter the results of the present study, which was restricted to changes occurring in the glandular epithelium. Second endometrial biopsies were taken on days LH+5, 6, 8 and 9. In the present study, four or five women were biopsied on each day.

Control biopsies

Some of the baseline biopsies, along with others taken from similar subjects who were not given RU486, were used to create a control group of 17 women (see Table I).

Biopsy procedure

Biopsies were taken as an outpatient procedure with or without Entonox analgesia. All biopsies were performed by one operator (T.C.L.) using a Sharman's curette. A single specimen was obtained from the fundus and upper part of the body of the uterus.

Histological processing

Histological processing is described in more detail in an earlier paper (Dockery *et al.*, 1988b). To summarize, the tissue was fixed in 2% glutaraldehyde and cut up in a standardized manner which yielded five pieces of tissue. Two large pieces of tissue were used for histological dating by light microscopy (Li *et al.*, 1988b) and immunocytochemistry (Graham *et al.*, 1991). The rest were processed for transmission electron microscopy.

In the present study, sections with a silver-grey interference colour (70 nm thick) were obtained from two blocks per biopsy, using a diamond knife on a Reichert OMU4 ultramicrotome. Sections were picked up on 200 mesh copper grids, stained with uranyl acetate and lead citrate and examined on a Phillips EM301 or CM10 electron microscope. Semi-thin sections were obtained from some of the biopsies and stained with Toluidine Blue. These sections were used for morphometric analysis.

Morphometric procedure for examination of glandular epithelium

This procedure has already been described in detail (Dockery *et al.*, 1988b, 1991). The volume of nuclei was estimated and combined with various cellular proportions to obtain cell and cytoplasmic dimensions. In the present study, these measurements were made on post-RU486 biopsies at days LH+5, +6, +8 and +9 ($n = 4$ in each group). These were combined with data from control biopsies ($n = 4$ or 5) in each group at corresponding days.

Statistical methods

Initially, each parameter was estimated for each individual, then the mean ± SD was calculated for each group. A two-way analysis of variance (ANOVA) was performed to examine the effect of day (of biopsy), treatment and interaction. A one-way analysis of variance followed by Tukey's test was then performed to examine differences between groups. The conventional probability of $P < 0.05$ was taken as the limit of statistical significance. All of the tests were performed using SPSS software on an IBM compatible PC.

Results

Qualitative findings for control biopsies

Control biopsies were in line with those described by Dockery *et al.* (1988b) and Cornillie *et al.* (1985) at day LH+5. The characteristic secretory triad was well developed in the control material, with giant mitochondrial profiles and glycogen deposits prominent in the basal cytoplasm. Nuclear channel systems were well developed. At LH+6, the secretory apparatus in the control material was now very elaborate. By LH+8, the secretory apparatus was not as well developed as before and small glycogen deposits were present throughout the cytoplasm. Intercellular interdigitations between adjacent epithelial cells were a prominent feature of the control cells. At LH+9/10, there was a degree of heterogeneity in cell morphology, with cuboidal and columnar cells seen. No nuclear channel systems were seen and the secretory apparatus was relatively poorly developed.

Qualitative findings for RU486-treated biopsies

In the present study, we found that a single variable dose of RU486 in the early luteal phase affected the secretory

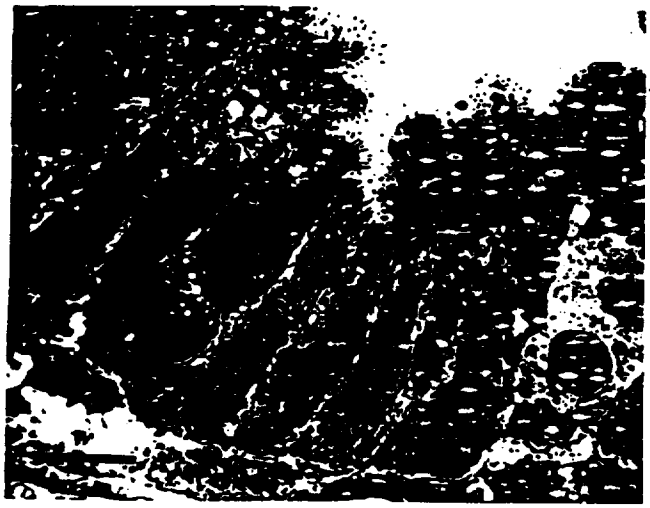

Figure 1. Glandular epithelial cells from RU486-treated biopsy at LH+5. Note lack of characteristic polarity and presence of degenerating cells (bar = 5 μm).

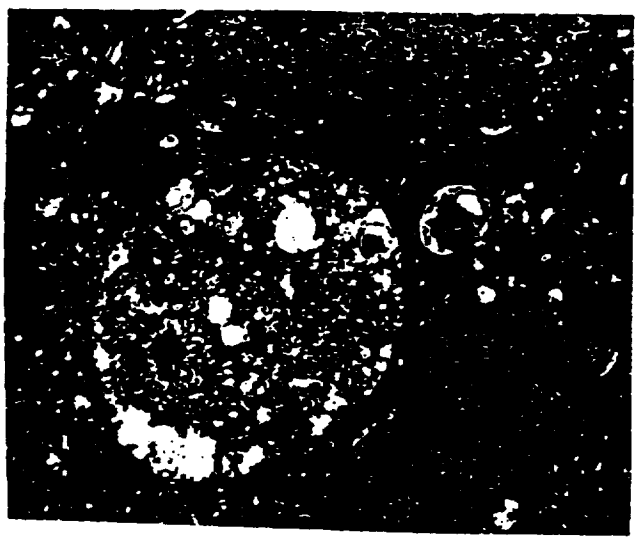

Figure 2. Degenerating cell in glandular epithelium of RU486-treated biopsy (LH+8) (bar = 1 μm).

machinery of the glandular epithelial cells; the picture was similar on all days studied. The cells were tall and columnar, and there was a loss of the highly polarized phenotype characteristic of control biopsies at days LH+5 and +6 (Figure 1).

The secretory apparatus was less developed than in the control biopsies, particularly at LH+5 and +6. Some cells appeared to have undergone complete cellular breakdown (Figures 1 and 2). These dead cells corresponded to the 'apoptotic bodies' described by Li *et al.* (1988b). Lysosome-like bodies were common in the apical and basal cytoplasm. Mitochondrial profiles were generally small and distributed throughout the cytoplasm (Figures 3 and 4). A few 'giant' mitochondrial profiles were present in only one biopsy, from a patient given a low dose of RU486. They were not confined to the basal portion of the cell (Figure 5). Occasional cells had small deposits of glycogen-rich material distributed throughout the cytoplasm (Figure 3). Electron-dense, lipid-like bodies were another feature of the RU486-treated cells (Figure 6).

Figure 3. Apical portion of gland cell from RU486-treated biopsy. Note small mitochondrial profiles (bar = 1 μm).

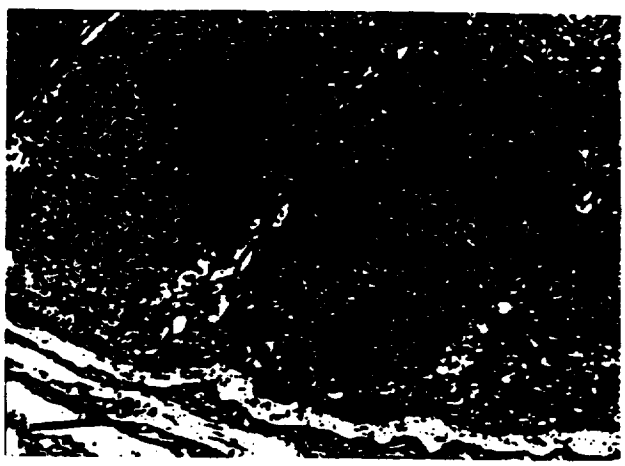

Figure 4. Basal portion of gland cell from RU486-treated biopsy at LH+6 (bar = 1 μm).

The nuclei were large and plump (Figure 7). Nuclear channels were present in only one RU486-treated biopsy at LH+5 (Figure 8). This biopsy exhibited a greater degree of polarity compared to other treated biopsies taken at this time.

With the relatively small numbers of subjects, it was not possible to state with confidence that the effects of RU486 were dose dependent. However, at the ultrastructural level, we found that the lower doses of 25 and 50 mg produced fewer effects than ≥100 mg. This was different from our earlier report based on a light microscopical assessment (Li *et al.*, 1988b).

Quantitative findings

Cell volume

A two-way ANOVA of cell volume data revealed no significant effect of day of biopsy, treatment or interaction (Tables II and III). At day LH+5, the cell volume of the control group was numerically larger than that of the treated group; this was reversed at day LH+9/10. In the control group, the mean cell volume decreased from 1237 μm^3 at LH+5 to 871 μm^3 by LH+9/10. These differences, however, were not statistically significant.

Figure 5. Apical portion of glandular epithelial cell from treated biopsy at LH+8. Note presence of giant mitochondrial profiles (bar = 1 μm).

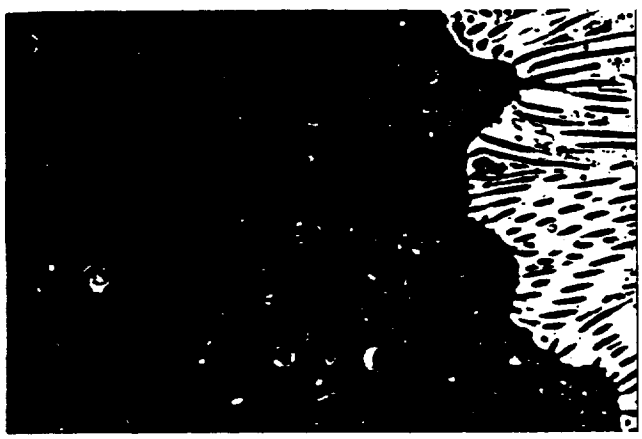

Figure 6. Characteristic electron-dense, lipid-like deposits in the apical cytoplasm of an RU486-treated biopsy (bar = 1 μm).

Nuclear volume

A two-way ANOVA of nuclear volume data also revealed no effect of day of biopsy, treatment or interaction (Tables II and III).

Nucleolar volume

A two-way ANOVA of nucleolar volume data revealed a significant effect of day of biopsy but no effect of treatment or interaction (Tables II and III); the nucleoli were found to be largest at day LH+5 in both groups and smallest at LH+9/10; this decrease represents 30% in the control group and 40% in the treated group.

Organelle volumes

Mitochondrial volume

A two-way ANOVA revealed a significant effect of day of biopsy and a significant interaction (Tables II and IV). The largest mean mitochondrial volume per cell was found in the day LH+5 control; this was significantly larger than all other biopsies.

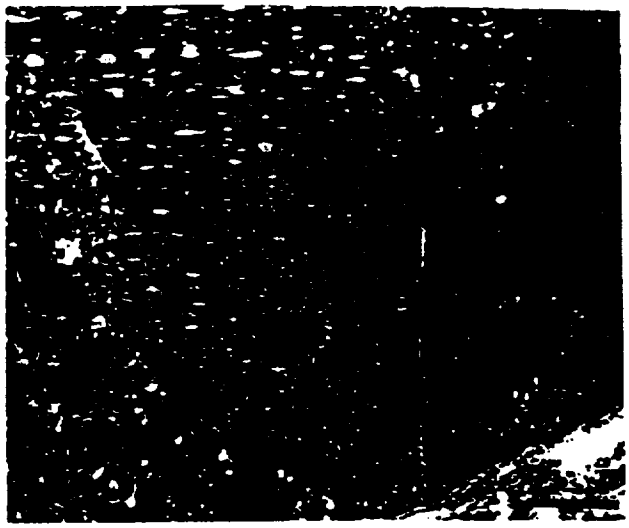

Figure 7. Nucleus of epithelial cell from RU486-treated biopsy at LH+8 (bar = 1 μm).

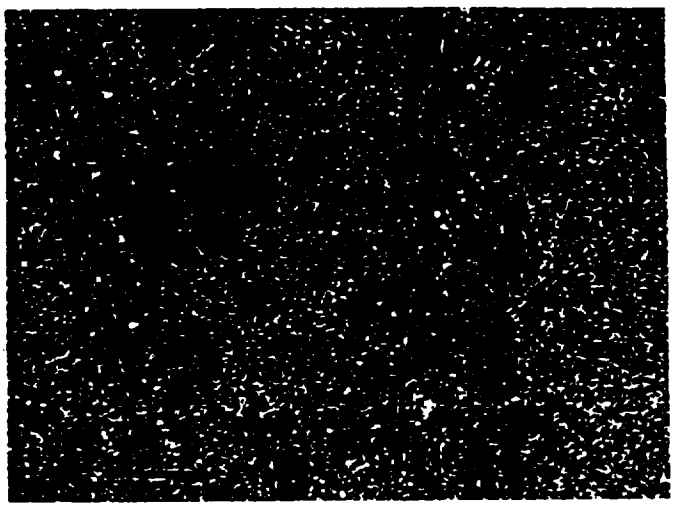

Figure 8. Two nuclear channel systems in nuclei of treated biopsy at LH+5 given 25 mg of RU486 (bar = 1 μm).

Glycogen volume

A two-way ANOVA revealed a significant effect of interaction but borderline effect of day of biopsy (Tables II and IV). The coefficient of variation [i.e. (SD×100)/mean] of this parameter was very large. The largest volume was found in the LH+5 control biopsies.

Rough endoplasmic reticulum volume

A two-way ANOVA revealed no significant effect of day of biopsy, treatment or interaction (Tables II and IV).

Secretory apparatus volume

A two-way ANOVA on the volume of secretory apparatus revealed a significant effect of day of biopsy, treatment and interaction (Tables II and IV). The volume of the secretory apparatus was significantly smaller in the treated groups than in the control groups at days LH+5 and +6.

Discussion

The administration of RU486 in the early luteal phase induces a delay in endometrial glandular secretory differentiation in

Table II. Two-way analysis of variance of volume comparisons in Tables III and IV

Feature	Day of biopsy (df = 3,33)		Condition (df = 1,33)		Interaction (df = 3,33)	
	F	*P*	*F*	*P*	*F*	*P*
Cell volume	1.43	NS	0.53	NS	1.06	NS
Nuclear volume	0.37	NS	3.56	NS	1.63	NS
Nucleolar volume	5.69	<0.01	0.74	NS	0.65	NS
Mitochondrial volume	9.99	<0.01	2.82	NS	3.28	<0.05
RER volume	0.64	NS	1.43	NS	0.42	NS
Glycogen volume	2.86	(<0.06)	0.39	NS	3.09	<0.05
Sec. volume	5.9	<0.01	12.86	<0.01	6.44	<0.01

df = degrees of freedom (numerator, denominator); NS = not significant; RER = rough endoplasmic reticulum; Sec = secretory apparatus.

Table III. Comparison of cell nuclear and nucleolar volumes (μm^3) at different times after the luteinizing hormone (LH) peak (LH+0), in control and RU486-treated subjects. Values represent mean ± SD

Day of biopsy	Feature	Control	RU486-treated
LH+5	nuclear volume	265.2 ± 18.9	255.9 ± 70.0
	cell volume	1236.9 ± 200.4	1090.9 ± 260.3
	volume nucleolus	7.2 ± 2.6	7.6 ± 2.3
LH+6	nuclear volume	260.8 ± 19.1	262.0 ± 73.2
	cell volume	1114.5 ± 192.9	1095.7 ± 364.7
	volume nucleolus	5.1 ± 1.8	6.7 ± 3.1
LH+8	nuclear volume	213.4 ± 54.8	307.4 ± 83.4
	cell volume	836.0 ± 190.6	1088.7 ± 368.8
	volume nucleolus	4.6 ± 0.3	5.9 ± 2.0
LH+9/10	nuclear volume	209.8 ± 32.5	266.6 ± 54.3
	cell volume	871.1 ± 151.4	1039.2 ± 244.9
	volume nucleolus	4.0 ± 1.0	3.1 ± 1.3

Table IV. Comparison of organelle volumes (μm^3) at different times after the luteinizing hormone (LH) peak (LH+0) in control and RU486-treated subjects. Values represent mean ± SD

Day of biopsy	Feature	Control	RU486-treated
LH+5	mitochondrial volume	96.9 ± 25.3	57.7 ± 31.9
	glycogen volume	142.1 ± 109.4	37.8 ± 33.8
	RER volume	23.9 ± 7.15	37.3 ± 35.6
	sec volume	35.9 ± 10.5	14.6 ± 4.2
LH+6	mitochondrial volume	41.0 ± 8.0	24.7 ± 14.4
	glycogen volume	17.1 ± 7.27	35.0 ± 26.4
	RER volume	36.4 ± 12.0	33.9 ± 23.9
	sec volume	41.7 ± 26.4	6.41 ± 3.1
LH+8	mitochondrial volume	38.9 ± 7.7	42.4 ± 12.3
	glycogen volume	19.9 ± 18.5	39.2 ± 42.6
	RER volume	23.3 ± 8.6	27.4 ± 12.0
	sec volume	7.4 ± 4.9	7.9 ± 3.2
LH+9/10	mitochondrial volume	40.4 ± 6.7	50.5 ± 18.5
	glycogen volume	24.9 ± 24.9	47.1 ± 46.2
	RER volume	18.4 ± 7.8	31.7 ± 13.5
	sec volume	9.5 ± 4.9	13.5 ± 4.9

RER = rough endoplasmic reticulum; sec = secretory apparatus.

the human (Li *et al.*, 1988b; Batista *et al.*, 1992; Gemzell-Danielsson *et al.*, 1996) and the rhesus monkey (Ghosh *et al.*, 1996). Graham *et al.* (1991), using the same material as in the present study, reported that when RU486 was administered on day LH+2 it prevented the appearance of the secretory glycan D9B1. However, administration on day LH+5 failed to prevent its expression. The present study provides an insight into the cellular machinery, including nuclear, nucleolar and secretory apparatus components, responsible for the production of endometrial secretions and how they are affected by progesterone blockade.

The secretory triad of cellular events that are characteristic of the early luteal endometrium (Cornillie *et al.*, 1985; Dockery and Rogers, 1989) was disrupted in the treated groups. RU486 seemed to affect the formation of the nuclear channel system and the mitochondrial elaboration which are characteristic of the human endometrium at day LH+5. If the drug was administered when these structures were present, it seemed to cause their disappearance from the cells. The administration of this compound appears to alter the dynamic sequence of events that occurs in the glandular epithelial cells during the early luteal phase. However, whether this is a direct effect of progesterone receptor blockade remains unknown.

In a recent study (Dockery *et al.*, 1996) on the nuclear channel system, we reported a delay in its development in women with unexplained infertility. These women also had a delayed expression of the secretory apparatus (Dockery *et al.*, 1993) which correlated with a delayed/reduced expression of the luteal-specific protein D9B1 (Graham *et al.*, 1990). The nuclear channel systems were seen in only one individual in the present study given a low dose (25 mg) of RU486 on day LH+2. This again seems to suggest that this progesterone-dependent organelle is intimately involved in the elaboration of the secretory apparatus. It is of interest to note that, in the study by Graham *et al.* (1991), early administration of RU486 prevented the expression of the secretory glycan, while later administration, when the machinery was in place, did not. From these observations it was suggested that the production of the secretory product is progesterone dependent and that secretion of material is progesterone independent. During the early luteal phase, the apical cytoplasm of the cells becomes packed with an elaborate secretory apparatus (Cornillie *et al.*, 1985; Dockery *et al.*, 1988b). Administration of RU486 before this machinery is in place prevents its formation, while administration of RU486 when the machinery is present seems to promote its breakdown. There appears to be a loss of the characteristic cytoplasmic polarity after RU486 treatment, which may suggest that progesterone or the affected organelles are important in maintaining this polarity.

Mitochondrial elaboration is a characteristic feature of the early luteal phase, perhaps to accommodate the high energy demands of the production of the secretory product. Mitochondrial volume is reduced on day LH+5 after RU486 administra-

tion, perhaps illustrating the progesterone-dependent nature of the expansion of this organelle.

The intimate association between semi-rough endoplasmic reticulum and mitochondrial profiles (Dockery *et al.*, 1988b) tends to be lost after administration of RU486. Another antiprogesterone compound, R2323, has been reported to have deleterious effects on both nuclear channels and mitochondria (Azadian-Boulanger *et al.*, 1971); the cytoplasmic events found in the present study were much more marked than those described for R2323.

The present study has reported that accumulation of lysosome-like structures is a notable feature of the apical cytoplasm after administration of RU486. Marked cellular degeneration is a notable feature of a subpopulation of the endometrial glandular cells. This would correspond to the increase in apoptosis described in the light microscopical observations of Li *et al.* (1988b). This feature has also been noted by Ghosh *et al.* (1996) in the rhesus monkey after luteal administration of RU486.

Some of the electron-dense structures have an appearance similar to the accumulation of lipid (Figure 6). Lipid accumulation is present in the cytoplasm from day LH+3 in normal endometrial glandular cells (Dockery and Rogers, 1989). The increased occurrence of these structures in the post-RU486 material perhaps implies that the cell no longer has the ability to mobilize this material.

The administration of RU486 does not simply retard endometrial development. It alters it in a quite distinctive manner. Many of the changes described appear to be regressive phenomena. This drug provides a unique opportunity to investigate the influence of progesterone on endometrial morphology.

The effect of a single dose of RU486 on the endometrium depends on when and how the drug is administered (Li *et al.*, 1988b,c; Johannisson *et al.*, 1989; Gemzell-Danielsson *et al.*, 1996); this may account for the discrepancies in the reported success of RU486 in early and late luteal phase contraception (Gemzell-Danielsson *et al.*, 1993; Van Look and von Hertzen 1995). Continuous daily administration of a low dose (1 mg) of RU486 produced retarded endometrial development in the luteal phase, similar to that seen in infertile women with luteal phase defect (Batista *et al.*, 1992). Longer term administration of RU486 at a higher dose (50 mg) produced changes which were similar to unopposed oestrogen therapy (Murphy *et al.*, 1995).

From this limited study it appears that endometrium treated with RU486 early in the luteal phase has substantial ultrastructural alterations which may interfere with the process of implantation. Whether the 'implantation window' is simply delayed is still a matter of controversy (Sarantis *et al.*, 1988).

We (Li *et al.*, 1988c, 1990) reported earlier that administration of RU486 in the luteal phase may induce menstruation, but that this may not be associated with the shedding of the functional layer of the endometrium. This may provide an explanation why in some cases of successful menstrual induction by RU486, pregnancy continued undisturbed (van Santen and Haspels, 1987). The ability of RU486 to interrupt a very early pregnancy is more likely to be related to its ability to cause shedding of the endometrium than to its ability to induce menstruation.

The present study has shown that RU486 disrupts the secretory events within the glandular epithelium. Perhaps the most marked feature of the glandular epithelial cells after the administration of RU486 was the loss of cytoplasmic polarity. This may imply that progesterone plays some role in generating and maintaining the highly polarized phenotype seen during the early luteal phase. If the drug is administered on day LH+4 or later, sufficient product may be present within the gland lumen to facilitate some support to the trophoblast (Graham *et al.*, 1991). If there is some sort of a safety net in stromal and glandular development, what role does the luminal epithelium play in permitting implantation?

By using electron microscopical morphometric methods, we have provided a unique insight into the progesterone-dependent nature of the structural reorganization of the glandular epithelium in the secretory phase of the menstrual cycle.

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The Effects of Mifepristone on Cervical Ripening and Labor Induction in Primigravidae

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Objective: To compare the effects of 50 mg or 200 mg of oral mifepristone with placebo on cervical ripening and induction of labor in primigravid women at term with unfavorable cervices.

Methods: This was a double-blind study in which 80 primigravidae at term with a modified Bishop score of 4 or less were randomly assigned to one of three treatment groups. They were assessed at 24-hour intervals for 72 hours, after which labor was induced if it had not occurred spontaneously.

Results: Two hundred milligrams of mifepristone resulted in a favorable cervix (with a Bishop score greater than 6 or in spontaneous labor) in significantly more women than placebo (P = .01). An improvement in cervical ripening was seen in the group given 50 mg of mifepristone, but this was not statistically significant. There were more cesarean deliveries performed for fetal distress in the group treated with 200 mg of mifepristone than placebo, but this was not statistically significant and was not associated with any differences between groups in terms of neonatal outcome.

Conclusion: Mifepristone, a progesterone antagonist, is known to cause softening and dilation of the human early pregnant cervix and an increase in uterine activity. It is theoretically attractive for use as an adjunct in cervical priming and labor induction. In this study, 200 mg of mifepristone was significantly more likely to result in a favorable cervix than placebo. (Obstet Gynecol 1998;92: 804–9. © 1998 by The American College of Obstetricians and Gynecologists.)

Induction of labor involves promoting softening and dilation ("ripening") of the cervix and producing effective myometrial contractions. It has been shown that induction is more likely to have a successful outcome if the cervix can be ripened before the onset of contractions, whether spontaneous or augmented.[1] Prostaglandin E_2 (PGE_2) is now widely used to prepare the unfavorable cervix for parturition.[1–3] However, prostaglandin preparations may cause uterine hyperstimulation[2] or may fail to produce sufficient cervical ripening for labor induction to proceed.

Mifepristone (RU 486, Hoechst-Roussel, Uxbridge, UK) is a potent progesterone and glucocorticoid antagonist acting on the progesterone receptor.[4] Its main clinical application has been as an abortifacient; however, it has been noted that its administration causes marked cervical softening in the first trimester, either in conjunction with prostaglandins promoting medical abortion[5] or when used alone before vacuum aspiration of the uterus.[6,7] Mifepristone shows synergism with prostaglandins in causing termination of pregnancy in the first or second trimester.[5,8] It seems reasonable, therefore, to propose mifepristone as an agent for cervical ripening in the third trimester, particularly in conjunction with prostaglandins. In primates, mifepristone has been demonstrated to be efficacious, in combination with oxytocin, in achieving cervical dilation and induction of labor.[9] In humans, Frydman et al[10] have shown that administration of 200 mg of mifepristone on 2 consecutive days to women at term significantly increased the number entering labor and decreased the prostaglandin requirements of the remainder, as compared with placebo.

Our study evaluated two doses of mifepristone (50 mg and 200 mg) as compared with placebo for their effect on cervical ripening and subsequent induction of labor in primigravid women whose cervices were initially unfavorable for induction. We also evaluated the maternal, fetal, and neonatal safety of mifepristone when used for this purpose. Because mifepristone is also a potent glucocorticoid antagonist[4] and because it is known to cross the placenta,[11,12] we also assessed whether there was an increased risk of neonatal hypoglycemia after its antepartum use.

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Materials and Methods

We conducted a placebo controlled double-blind trial in which two single oral doses (50 mg and 200 mg) of mifepristone were compared with placebo in a dose escalation study to assess their efficacy and safety for the induction of labor in primigravid women at term. In the first part of the study, the efficacy of a dose of 50 mg of mifepristone was compared with placebo. On the basis of an interim efficacy analysis of this dose compared with the placebo, we then determined whether a higher or a lower dose of mifepristone should be used in the second part of the study. Twenty-five women were included in each treatment arm in the first part. On the basis of the interim efficacy analysis, an increased dose of 200 mg was used in the second part of the study. In the second part of the study, 25 women received the higher dose of mifepristone and another five were randomly assigned to receive the placebo (the placebo groups from both parts of the study were pooled for the final analysis). The sample size was calculated to detect a 40% difference in the number of patients who went into spontaneous labor or who had a Bishop score greater than or equal to 6 comparing patients receiving mifepristone and patients receiving placebo with a power of 90% at the 5% significance level. Randomization was achieved by predetermined randomization code. Patients were allocated a number, and therefore treatment, in strict numeric order as they entered the study. The study was approved by the Lothian Research Ethics Reproductive Medicine Subcommittee.

The inclusion criteria for the study stated that patients were primiparous women aged 18 to 40 years with a normal, live, single, cephalic presentation. Gestation length was between 37 and 41 weeks 4 days as determined by a first-trimester ultrasound scan. Labor induction was scheduled 72 hours after treatment. Patients were offered the option of inclusion in the study if they had a modified Bishop score[13] of 4 or less. After written informed consent was obtained, neither the patient nor the physician had knowledge of whether a single oral dose of mifepristone or placebo was given. Patients were excluded if they showed signs or symptoms of the onset of labor, of placental insufficiency, or if they had any contraindication to the use of mifepristone. Before study medication was given, a modified Bishop score was assigned by vaginal examination. In most women, subsequent cervical assessments were performed by the same investigator, each of whom was blinded to the treatment allocations.

After entering the study, the women were examined as outpatients after 24 and 48 hours. Fetal wellbeing was assessed by the women keeping a 'kick-chart' for each 24-hour period and by a further cardiotocograph at each review visit. At the 24- and 48-hour reviews maternal blood pressure and pulse were recorded, the vaginal examination was repeated, and a Bishop score calculated.

If labor had not occurred within 72 hours after taking the allocated treatment the woman was admitted for induction. An initial dose of 1 mg of PGE_2 gel was inserted into the posterior fornix of the vagina unless the cervix was more than 3 cm dilated and fully effaced, in which case artificial rupture of the membranes was performed. The cervix was reassessed after 6 hours and (unless labor was established) a further dose of 1 or 2 mg of PGE_2 was given intravaginally. The examination was repeated every 4 hours, and when appropriate, artificial rupture of the membranes was performed. Oxytocin was administered as clinically indicated. The fetal heart rate was monitored continuously once labor was established.

A cord blood sample was obtained at delivery for blood gas analysis, biochemical and hematologic factors, and for assay of cortisol, ACTH, and mifepristone levels.

Neonatal blood glucose was monitored 1, 3, and 12 hours after delivery by means of a heel prick sample. Neonatal weight, pulse, and temperature were recorded after 24 and 48 hours, as were any adverse clinical findings in the mother or infant. A further sample of maternal blood was taken 24 hours after delivery. Both mother and baby were reviewed 1 week and 1 month after delivery; standard observations were recorded, and any adverse events were noted.

Data were stored on computer database, and analysis was performed using the Statistical Analysis Software (SAS) package (SAS Institute, Cary, North Carolina). Differences between the treatment groups were compared using the t test for continuous data and the χ^2 test for categoric data.

The primary efficacy measure was successful cervical ripening (ie, modified Bishop score of 6 or greater or the onset of spontaneous labor) within 72 hours of treatment administration.

Results

The patient characteristics are given in Table 1. One woman was 17 years old at inclusion into the study and one was at a gestation of 41 weeks and 5 days when she received treatment. Although these constituted violations of the protocol, both were included in the efficacy analysis. Proportional odds models were fitted to the data, with and without factors for maternal age, gestational age, weight gain, weight at booking, and pretreatment Bishop score. None of these factors were found to

Table 1. Patient Characteristics

Characteristic	Placebo	Mifepristone 50 mg	Mifepristone 200 mg
Age (y), mean ± SD	26.2 ± 5.9	25.8 ± 4.5	25.6 ± 3.3
Gestation (wk + d), mean (SD d)	40 + 6 (3.6)	40 + 5 (5.5)	40 + 6 (5.1)
Booking weight (kg), mean ± SD	62.2 ± 8.9	71.8 ± 13.6	69.8 ± 13.3
Initial cervical score, median (range)	3 (1–4)	4 (2–4)	3 (1–4)
Previous pregnancy			
First trimester termination	2	1	4
First miscarriage	4	5	1
Ectopic	0	1	0

SD = standard deviation.

be statistically significant at the 5% level and were excluded from the final model.

In all three treatment groups the most common reason for inclusion was prolonged pregnancy (longer than 40 weeks). The next most common reason was hypertensive disorders.

The main outcome measures are summarized in Table 2. Cervical ripening was deemed successful if spontaneous labor had ensued or if the Bishop score was greater than 6 before induction of labor 72 hours after the treatment administration. Cervical ripening was successful for 64%, 48%, and 30% of the patients treated with 200 mg, 50 mg of mifepristone, and placebo, respectively. This difference was statistically significant for the 200 mg group (P = .01, odds ratio (OR) 4.15, 95% confidence interval (CI) 1.34, 12.84). There was no statistically significant difference between the 50-mg mifepristone group and the placebo group (P = .18, OR 2.36, 95% CI 0.66, 8.37). The number of patients in spontaneous labor after 72 hours was 9 (36%), 8 (32%), and 7 (23.33%) in the 200-mg, 50-mg and placebo groups, respectively.

The subsequent course of labor is summarized in Table 3 and the modes of delivery in Figure 1. Similar numbers of women in all three groups were given PGE_2, had an artificial rupture of membranes, and received oxytocin. The median amount of oxytocin required was 1095 mU, 5198 mU, and 5780 mU for the 200-mg, 50-mg, and placebo groups, respectively. This reduction in the requirement by the former group is not statistically significant. Significantly fewer women who received 50 mg of mifepristone had a cesarean delivery than those who were given placebo (P = .033, χ^2 = 4.55); however, in the group given 200 mg of mifepristone there was no statistically significant difference from the placebo group in the overall number requiring cesarean delivery (P = .075). In the 200-mg treatment group, eight of nine cesareans were performed for fetal distress and the other for failure to progress. In the placebo-treated group, three of eight cesareans were for distress and five for failure to progress.

Table 2. Outcome at 72 Hours After Treatment

	Placebo (n = 30)	Mifepristone 50 mg (n = 25)	Mifepristone 200 mg (n = 25)
Spontaneous labor	7 (23.3)	8 (32)	9 (36)
Induced with a BS ≥6	2 (6.7)	4 (16)	7 (28)
Successful cervical ripening*	9 (30)	12 (48)	16 (64)†

BS = Bishop score.
Data are given as number (%).
* Successful cervical ripening was defined as Bishop score greater than 6 or being in spontaneous labor.
† P = .01 compared with placebo.

A wide range of maternal minor adverse events were reported in all treatment groups, most of which were complications of pregnancy, such as hemorrhoids or headache. There were no marked differences across the groups in the reporting of these. One patient in the 200-mg mifepristone treatment group had transiently increased liver function tests.

There were no episodes of antepartum fetal distress after recruitment to the study. In labor, fetal distress was considered severe if it required medical intervention (such as fetal blood sampling) or delivery. There were 12 (48%), 6 (24%), and 4 (13.3%) cases of fetal distress in the 200-mg mifepristone, 50-mg mifepristone, and placebo groups, respectively.

Blood was taken from the umbilical vein at delivery. Because of practical problems, this was not possible for all infants in the study. The blood gas levels, ACTH, and cortisol values from these samples are summarized in Table 4. There were no statistically significant differences between the groups in any of the cord gas levels. The analysis of ACTH and cortisol in the cord blood showed a wide range of values; the values for ACTH, in particular, show wide standard deviation. There was no significant difference between the 200-mg mifepristone group and the placebo group. The mean levels of mifepristone in the cord blood were .048 mg/L (standard deviation [SD] = .038) and .054 mg/L (SD = .047) after 50 mg and 200 mg of mifepristone, respectively. Figure 2 illustrates the relationship between the length of time to delivery after mifepristone administration and the cord blood levels of mifepristone. The Apgar scores of the infants were recorded at 1 minute and 5 minutes in all of the infants and at 10 minutes in eight infants. There were no significant differences in these scores across the treatment groups.

Hypoglycemia in the neonates was monitored after delivery by means of a heel-prick sample at 1, 3, and 12

Table 3. Course of Subsequent Labor

	Placebo (n = 30)	Mifepristone 50 mg (n = 25)	Mifepristone 200 mg (n = 25)
Time to onset of labor, median	81 h 15 min	80 h 20 min	75 h 50 min
Range	(17 h 40 min to 104 h 30 min)	(6 h 55 min to 100 h)	(9 h 45 min to 101 h 15 min)
Time to delivery, median	88 h 14 min	85 h 15 min	84 h 6 min
Range	(27 h 21 min to 113 h 35 min)	(15 h 12 min to 113 h 47 min)	(13 h 1 min to 110 h 49 min)
PGE_2 given	23 (76.7%)	17 (68%)	16 (64%)
Total median PGE_2 dose (mg)	3	3	3
Oxytocin administered	14 (46.7%)	8 (32%)	12 (48%)
Total oxytocin dose (mU), median (range)	5780 (210–19,650)	5198 (2060–20,800)	1095 (210–14,220)

PGE_2 = prostaglandin E_2.

hours after delivery. Hypoglycemia was defined as a reading of less than 2.2, which was the case in a total of 10, 11, and 10 infants of mothers who received 200 mg of mifepristone, 50 mg of mifepristone, and placebo, respectively. Only one infant in the study population was admitted to the Special Care Unit as a result of hypoglycemia; the mother of this infant had received placebo. Figure 3 illustrates the mean glucose levels in the neonates.

Neonatal jaundice was reported in seven (28%), two (8%), and two (6.7%) of the infants of mothers who received 200 mg of mifepristone, 50 mg of mifepristone, and placebo, respectively. All of these cases resolved spontaneously and were not considered clinically significant.

Discussion

Mifepristone was deemed to have successfully promoted cervical ripening in primigravid women at term with an initially unfavorable cervix if after 72 hours the woman was in spontaneous labor or the cervix had become favorable (Bishop score greater than 6). By these criteria, 200 mg of mifepristone had a significant effect on cervical ripening, whereas the effect of 50 mg was just below the level of statistical significance compared with the placebo. An improvement in the Bishop score confers an increased chance of successful labor induction[13] which may be of particular benefit to this group of women. These findings confirm those of Frydman et al[10] who gave 200 mg of mifepristone on 2 consecutive days to women at term. As the effect of 50 mg of mifepristone in our study just failed to reach significance levels, it seems likely that a slightly increased dose, such as 75 mg or 100 mg, may also significantly

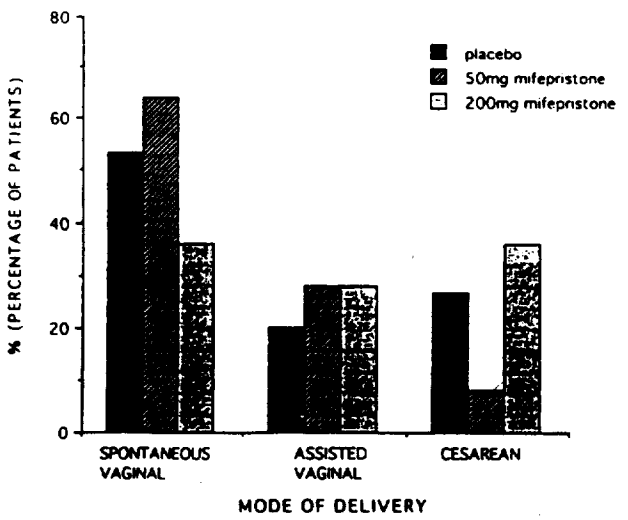

Figure 1. Mode of delivery after treatment with 200 mg, 50 mg of mifepristone, or placebo.

Table 4. Umbilical Vein Blood Parameters

	Placebo (n = 30)	Mifepristone 50 mg (n = 25)	Mifepristone 200 mg (n = 25)
po_2 (mmHg)	n = 6	n = 6	n = 12
Mean (SD)	32.3 (11.0)	30.3 (6.0)	38.9 (36.5)
Range	22.5–53.3	24.0–39.8	9.0–137.3
pco_2 (mmHg)	n = 6	n = 6	n = 12
Mean (SD)	39.5 (96)	40.9 (40)	41.4 (10.5)
Range	24.0–52.0	34.3–45.3	13.1–52.0
pH	n = 16	n = 21	n = 14
Mean (SD)	7.3 (0.1)	7.3 (0.1)	7.3 (0.1)
Range	6.9–7.4	6.9–7.4	7.1–7.4
Base excess (mmol/L)	n = 5	n = 6	n = 12
Mean (SD)	−4.7 (5.8)	−5.3 (2.1)	−7.7 (5.5)
Range	−14.6 ± −0.7	−7.9–−2.3	−22.2–−1.7
ACTH (mU/L)	n = 25	n = 21	n = 19
Mean (SD)	51.5 (91.5)	41.8 (43.9)	20.7 (22.6)
Range	3–433	3–208	3–84
Cortisol (mmol/L)	n = 24	n = 21	n = 19
Mean (SD)	484.5 (223.2)	498.6 (158.9)	522.1 (190.2)
Range	234–1037	295–894	211–885

po_2 = partial pressure of oxygen; SD = standard deviation; pco_2 = partial pressure of carbon dioxide; ACTH = adrenocorticotropic hormone.

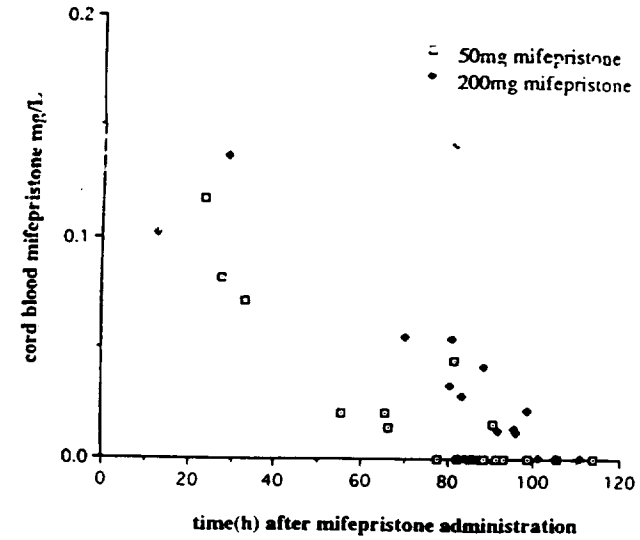

Figure 2. Mifepristone levels (ng/mL) in cord blood obtained at the time of delivery compared with the time to delivery after administration of 50 mg or 200 mg of mifepristone.

ripen the cervix and that doses greater than 200 mg could have a more pronounced effect.

As pregnancy progresses mifepristone becomes less efficacious at inducing abortion but its action in promoting cervical ripening is maintained.[4] This effect has been noted previously in Rhesus monkeys, in whom mifepristone alone induced cervical ripening but did not produce sufficient myometrial activity to affect delivery.[14] In combination with oxytocin, however, mifepristone was effective at inducing delivery in these monkeys.[9] As shown by this study, the most pronounced effect of mifepristone in the third trimester was in causing cervical ripening and increased sensitivity to oxytocin rather than labor induction. Women in this

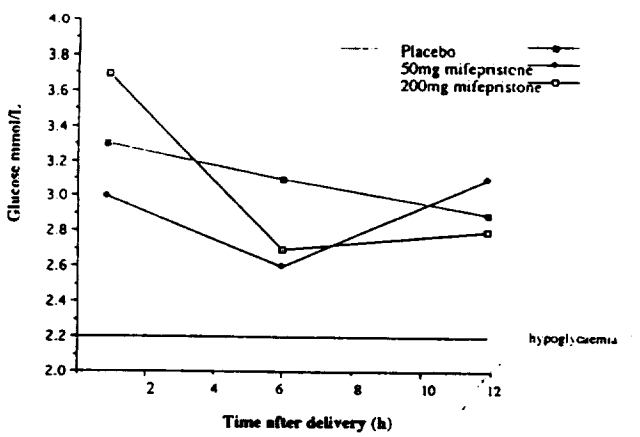

Figure 3. Mean neonatal blood glucose levels 1, 3, and 12 hours after delivery following antenatal administration of 50 mg or 200 mg of mifepristone.

study were initially given only 1 mg of PGE_2 after treatment administration because of concerns that increased uterine responsiveness and synergism between the two agents could lead to a risk of hyperstimulation. However, there were no episodes of hyperstimulation reported in any group.

The cervical changes after mifepristone administration have clinical implications. In addition, this finding may help to elucidate the processes whereby cervical ripening and the onset of labor occur spontaneously. The effect of antiprogesterones in causing cervical softening indirectly supports the role of progesterone in the maintenance of pregnancy.

In addition to efficacy, the other main outcome measures of the study were fetal and neonatal safety. Mifepristone has already been used extensively in women at higher doses than used here without major concerns regarding its safety. Our data showed no serious maternal side effects. Previous studies of placental transfer in the late second and third trimester of pregnancy in monkeys[11] showed that a steady-state equilibrium was relatively quickly established with a gradient between mother and fetus possibly limiting flux. It was also noted that the efficiency with which mifepristone crossed the placenta was significantly decreased in the third trimester compared with the second trimester of pregnancy. In the present study there was no evidence of altered glucocorticoid or mineralocorticoid action in the cord blood values obtained. As would be expected, the levels of mifepristone in the cord blood samples were higher in the group treated with 200 mg of mifepristone than in the group given 50 mg. The level of mifepristone in cord serum is inversely correlated with the length of time after administration that delivery occurs.

Fetal and neonatal wellbeing were assessed at each stage of the study. There was no antepartum evidence of fetal distress after administration of mifepristone, as assessed by cardiotocograph and by maternal record of fetal movement. Intrapartum distress was assessed clinically and was more often diagnosed in the women who received 200 mg of mifepristone than in women in the other two groups. In the 200-mg group, there were more cesarean deliveries for fetal distress and fewer for failure to progress, although there was no overall increase in the number of cesareans performed. In the group of women who received 50 mg of mifepristone there were fewer cesarean deliveries. No difference was found in cord pH values or Apgar scores between the three groups. Although this finding relates to small numbers of women, the possibility of increased fetal distress after treatment with 200 mg of mifepristone should be carefully evaluated in a larger trial before its use is recommended. In each group that received mife-

pristone, there was a decreased incidence of women requiring cesarean delivery as a result of failure to progress in labor as compared with placebo. In contrast to monkeys, labor induction after mifepristone administration in humans may result in a more efficient process, thus decreasing dystocia.

A dose of 200 mg of mifepristone had a significant effect on cervical ripening in this group of women, but it was associated with an increase in clinically suspected fetal distress, an effect not found with 50 mg of mifepristone. An intermediate dose may provide benefits in terms of cervical ripening without affecting fetal well-being. A larger trial is needed to ascertain more fully these effects.

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ORIGINAL RESEARCH ARTICLE

ELSEVIER

Side Effects of Mifepristone Misoprostol Abortion Versus Surgical Abortion

Data From a Trial in China, Cuba, and India

Batya Elul,* Charlotte Ellertson,† Beverly Winikoff,* and Kurus Coyaji‡

Although serious adverse events of early abortion have been studied, little attention has been paid to the more common side effects experienced by early medical or surgical abortion clients. Using data from a multicenter comparative trial of women ≤56 days' gestation in China, Cuba, and India (n = 1373), side effects experienced by mifepristone-misoprostol medical abortion and surgical abortion clients were analyzed at the different stages of their abortions. Data on side effects came from women's reports at each clinic visit, providers' observations during the clinic visits, and symptom diaries maintained during the study period. Medical abortion clients at all sites experienced more side effects than their surgical counterparts. The disparity between the two groups was particularly pronounced for bleeding and pain. Despite more reports of side effects among medical abortion clients, however, assessments of well-being and reports of satisfaction at the exit interview were similar in both treatment groups. CONTRACEPTION 1999;59:107–114 © *1999 Elsevier Science Inc. All rights reserved.*

KEY WORDS: side effects, early abortion, mifepristone-misoprostol, surgical abortion, medical abortion

Introduction

Over the past decade, several safe and effective medical abortion regimens have been developed.[1–3] The most widely used regimen consists of 600 mg mifepristone followed two days later by 400 μg oral misoprostol. This regimen has been available in France for >6 years,[1] received "approvable status" from the United States Food and Drug Administration in 1996[4] and has been tested in several developing countries as well.[5,6] Despite varied experiences with the method across different cultures, women consistently report the method's side effects, including bleeding and pain, among its worst features.[6–8]

Much of the existing literature on side effects of medical abortion is limited to serious adverse events or to a cursory mention of the more common side effects. Indeed, aside from four articles investigating blood loss experienced with various mifepristone regimens,[9–12] no thorough analysis of the method's side effects, including when they occur during the abortion process, how they affect patient acceptability, and how they compare with those experienced in connection with surgical abortion, has been undertaken. Yet, these factors typically govern women's experiences as much as the rare serious adverse event. In some cases, in fact, side effects of medical abortion are considered so unpleasant or severe as to negate the advantages the method offers over surgical alternatives. Indeed, women who initially select medical abortion in preference to surgical abortion may even request a surgical intervention to halt these side effects.[13]

Using data from a comparative trial of medical and surgical abortion, women's experiences with side effects were investigated in three developing countries. A fuller exposition of this topic is in order for several reasons, particularly if based on an international, multicenter, acceptability and feasibility comparative trial. First, as access to medical abortion is increased, side effects must be well understood so that proper training and counseling materials can be developed. Realistic expectations are essential for the method to be used by suitable providers and clients, and to minimize anxiety and unnecessary surgical interventions once treatment begins. Second, as regulatory decisions and clinical protocols for medical abortion are typically based in part on the side effect profile of the method, accurate documentation of the severity and incidence of side effects is critical, as is a context for understanding this profile. Third, reporting of side effects and reactions to them vary across cultures. Assessing women's experiences with side effects and their acceptability in three countries si-

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multaneously can highlight commonalties and differences. A nuisance side effect in one population may be entirely unacceptable in another. Finally, the medical literature on side effects experienced with surgical abortion is scanty, particularly with respect to the experiences of women in developing countries and in comparison with new medical abortion regimens. Indeed, protocols for managing side effects, such as pain, may differ greatly in developed and developing countries, contributing to a very different user experience with the two methods. A comparative analysis using data from three developing countries will help build this literature.

Materials and Methods

Data were analyzed from a large international comparative study of mifepristone-misoprostol medical abortion and surgical abortion held in China, Cuba, and India. Six urban clinics participated in the study, which ran from October 1991 to August 1993. All clinics already offered legal first-trimester surgical abortion services and followed a uniform study protocol approved by the Population Council's Institutional Review Board. Women with pregnancy durations of ≤56 days (based on the onset of the last menstrual period, bimanual examination, and, typically, ultrasound) could enroll if they were eligible* for either surgical or medical abortion. Eligible women received detailed explanations of the two methods and then selected one.† Women who could not decide were randomized to a method. All women gave informed consent.

Medical abortion clients received 600 mg mifepristone orally on their first visit and were observed for 30 min afterwards. At the second visit, 48 h later, women received 400 μg oral misoprostol and remained in the clinic under observation for 4 h. Rh-negative clients also received Rh_o(D) immune globulin at this visit. After 14 days, women returned for the final follow-up visit and exit interview. Women who did not have complete abortions by the time of the final visit received surgical interventions. All women who required or requested (for whatever reason) surgical interventions at any point before the end of the study also received them.

Surgical abortion clients received their abortions on the first visit according to standard clinical practices in each site. In Cuba, virtually all women received general anesthesia for dilation and sharp curettage. In India, women received light general anesthesia for vacuum aspiration. In China, in most cases, curettage was performed with no anesthesia at all, although a few women received local anesthesia. Clients returned after 14 days for follow-up examinations and exit interviews.

Using standardized questionnaires, providers gathered clinical and qualitative data at each clinic visit. Women were asked (both prompted and unprompted) about side effects at each visit, and providers also noted symptoms that women experienced during those visits. Women also completed symptom diaries in which they marked any symptoms (selected from a range) they experienced on each study day. Additionally, at their exit interviews, women answered a number of questions about method satisfaction and compared some of their side effects (ie, pain and bleeding) with prior expectations. Data were entered and analyzed at the Population Council using SPSS software. Statistical significance was set at $p \leq 0.05$. Levene's tests were employed to determine whether pooled or separate estimates of variance were appropriate in the t tests. Count data were analyzed using Fisher's exact tests or Pearson's χ^2 tests. All tests were two-tailed.

The main efficacy, safety, and acceptability results are reported elsewhere,[5,6] as is a separate analysis of bleeding, a particularly important and potentially dangerous side effect of mifepristone-misoprostol treatment.[12] This article, however, describes in detail the full range of side effects experienced by women during the various time intervals of their abortion procedure or process and compares them across methods and countries. Unlike other reports of side effects related to abortion, which typically present an overall incidence from the treatment period, we separated those side effects that occurred for medical abortion clients between the first visit (when mifepristone was ingested) and the second visit, 2 days later (when women returned for misoprostol), from those side effects occurring between the second visit and the third visit, 14 days later (when women returned for their follow-up visit). In the case of surgical abortion, we separated side effects that occurred on the day of the procedure from those experienced in the subsequent 14 days.

Results

Sample Characteristics and Failure Rates

Table 1 provides characteristics of the 1373 participants, as well as failure rates, by site, and method. "Failure" is defined as referring to any medical client

* Eligibility requirements were as follows: ≤56 days since last menstrual period (LMP); hemoglobin levels ≥10 g/dL; <35 years of age; smoke <10 cigarettes/day; could return for follow-up visits; did not want concurrent sterilization or IUD insertion; and not suffering from asthma or spasmodic bronchitis, cardiac disease, renal, hepatic or adrenal insufficiency, clotting disorders, or diabetes.
† Unfortunately, this part of the protocol was not implemented perfectly, and some sites enrolled women who were not eligible for both methods because they wanted concurrent IUD insertion, an option available only with surgical abortion.

Table 1. Characteristics of trial participants, by method and country

	Medical	Surgical
China (n)	299	268
Mean age (years)	27.6	27.9
Mean weight (kg)	53.2	53.6
Mean height (cm)*	162.3	161.5
Mean education (years)*	12.8	12.4
Mean gestation (weeks)*	5.5	6.2
Primigravida (%)	19.4	20.9
Married/in union (%)	91.0	91.0
Previously contracepting (%)	45.5	51.1
Previously aborted (%)*	70.6	56.7
Failure rate (%)	8.6	0.4
Assigned to method (n)†	3	52
Cuba (n)	250	249
Mean age (years)	23.3	23.6
Mean weight (kg)	54.2	54.8
Mean height (cm)*	162.8	162.0
Mean education (years)*	13.6	12.6
Mean gestation (weeks)*	6.5	7.1
Primigravida (%)	35.2	32.5
Married/in union (%)	41.4	49.4
Previously contracepting (%)	50.4	51.8
Previously aborted (%)	57.2	56.6
Failure rate (%)	16.0	4.0
Assigned to method (n)†	2	126
India (n)	250	57
Mean age (years)	25.5	25.1
Mean weight (kg)	47.1	45.5
Mean height (cm)	153.7	152.3
Primigravida (%)*	8.8	0.0
Married/in union (%)	99.2	100.0
Previously contracepting (%)	32.0	19.3
Previously aborted (%)	20.0	21.1
Mean education (years)*	10.4	9.1
Mean gestation (weeks)*	6.6	6.8
Failure rate (%)	5.2	0.0
Assigned to method (n)†	0	1

*Difference between medical and surgical groups is significant at $p \leq 0.05$ as determined by χ^2 tests.

†Women undecided between medical and surgical abortion were randomly assigned to a method. Additionally, because of the high demand for medical abortion among eligible participants and the consequent imbalance between samples in the two study arms, Chinese investigators enrolled women requesting IUD (and consequently ineligible for medical abortion) into the surgical group. Similarly, Cuban investigators withheld the medical option on certain days and consequently "assigned" women to the surgical arm.

Table 2. Baseline symptoms reported as ever occurring before treatment, and side effects experienced in 30-min periods after mifepristone administration (medical clients) or in recovery period after surgical procedure (surgical clients), by method and country (%)

	Medical	Surgical
Baseline symptoms		
China (n)	299	268
Nausea*	39.5	56.0
Vomiting*	1.7	14.6
Other	15.7	16.4
Cuba (n)	250	249
Nausea	18.0	14.1
Vomiting	6.4	8.4
Other*	4.0	0.8
India (n)	250	57
Nausea*	34.4	17.5
Vomiting	22.4	15.8
Other	17.2	12.3
After mifepristone or after surgical procedure		
China (n)	299	268
Vomiting*	16.7	83.3
Hemorrhage	0.0	0.7
Pain*	0.0	65.3
Other	6.0	9.7
Cuba (n)	250	249
Vomiting	0.4	2.8
Hemorrhage	0.4	0.0
Pain*	0.0	3.6
Other	0.0	0.0
India (n)	250	57
Vomiting*	1.2	0.0
Hemorrhage	0.0	0.0
Pain	0.0	0.0
Other	0.8	1.8

*Difference between medical and surgical groups is significant at $p \leq 0.05$ as determined by χ^2 tests.

who received a surgical intervention (whether on request, deemed medically necessary during the study, or for an ongoing pregnancy or incomplete abortion at the study end), and any surgical client who received a second surgical intervention. In all sites, surgical abortion was more effective than medical abortion. Sites that had high medical failure rates also tended to have high surgical failure rates.[5]

Side Effects

BASELINE SYMPTOMS. Typically, some of the side effects commonly attributed to medical or surgical abortion regimens are symptoms of the underlying condition (ie, pregnancy) being treated. For this reason, Table 2 presents the baseline symptoms women experienced before they began their treatment. These varied across countries and methods. In China, significantly more surgical than medical clients reported both nausea (medical 39.5% vs surgical 56.0%) and vomiting (1.7% vs 14.6%) before the admission visit. In Cuba, baseline rates of nausea (18.0% vs 14.1%) and vomiting (6.4% vs 8.4%) did not differ significantly by treatment group. In India, reports of vomiting before study admission were the most frequent for both methods (22.4% vs 15.8%), and baseline nausea differed significantly by method (34.0% vs 17.5%).

SYMPTOMS IMMEDIATELY AFTER THE INGESTION OF MIFEPRISTONE (MEDICAL CLIENTS ONLY) OR AFTER THE SURGICAL ABORTION (SURGICAL CLIENTS ONLY). Providers noted the side effects that women experienced in the 30 min after they took mifepristone or in the immediate

Table 4. Side effects experienced between last treatment visit and exit interview, by method and country (%)

	Medical	Surgical
China (n)	299	268
Nausea	6.0	3.7
Vomiting	1.3	0.4
Cramping/pain	20.1	19.4
Diarrhea	0.3	1.5
Profuse bleeding*	8.7	1.9
Prolonged bleeding*	41.1	9.0
Other	18.1	20.5
Cuba (n)	249	246
Nausea*	16.5	8.9
Vomiting*	7.6	3.3
Cramping/pain*	63.1	53.3
Diarrhea*	2.0	0.0
Profuse bleeding*	31.7	5.7
Prolonged bleeding*	71.1	21.1
Other*	19.7	52.4
India (n)	249	57
Nausea	10.4	3.5
Vomiting	6.8	5.3
Cramping/pain	28.9	24.6
Diarrhea	0.8	0.0
Profuse bleeding	10.8	3.5
Prolonged bleeding*	23.3	7.0
Other	25.3	24.6

*Difference between medical and surgical groups is significant at $p \leq 0.05$ as determined by χ^2 tests.

tol administration and before the exit interview was the longest period of time in which they managed side effects without assistance.

At the exit visit, cramping, profuse bleeding, and prolonged bleeding were the side effects most frequently reported since the last clinic visit for women in both treatment groups at all sites (Table 4). Profuse and prolonged bleeding, however, were reported by significantly higher proportions of medical than of surgical abortion clients in all countries, except in India where rates for the former did not differ significantly by method. Whereas cramping was reported by more medical clients (China 20.1%, Cuba 63.1%, India 28.9%) than surgical clients (China 19.4%, Cuba 53.3%, India 24.6%), this difference was significant only in Cuba. Indeed, in Cuba, every precoded side effect (ie, nausea, vomiting, cramping/pain, diarrhea, profuse bleeding, prolonged bleeding) was significantly more common among the medical clients than among the surgical clients. Surgical clients in Cuba, however, reported significantly more "other" side effects. Despite concerns about gastrointestinal side effects, very few women, regardless of their treatment modality, reported vomiting (<8.0%) and even fewer reported diarrhea (<2.0%). The likelihood of surgical clients in any country reporting side effects, including cramping and vomiting, since their last clinic visit was unrelated to the type of anesthesia used during the procedure.

Table 5. Side effects reported by women on daily charts as ever occurring during study period, by method and country (%)

	Medical	Surgical
China (n)	299	268
Gastrointestinal		
Nausea*	55.1	10.0
Vomiting*	23.3	1.5
Bleeding		
Spotting/light (<menses)	92.9	95.5
Normal (=menses)*	97.0	61.1
Heavy (>menses)*	52.2	4.2
Other		
Pain/cramps*	60.3	36.0
Fever	7.0	3.8
Physical restrictions*	26.5	12.6
Cuba (n)	250	182
Gastrointestinal		
Nausea*	70.4	19.2
Vomiting*	44.8	10.4
Bleeding		
Spotting/light (<menses)	84.0	79.1
Normal (=menses)*	90.0	65.4
Heavy (>menses)*	77.6	16.5
Other		
Pain/cramps*	89.2	65.4
Fever	9.2	7.1
Physical restrictions*	37.6	33.0
India (n)	247	57
Gastrointestinal		
Nausea*	56.5	7.0
Vomiting	39.3	12.3
Bleeding		
Spotting/light (<menses)	81.4	61.4
Normal (=menses)*	88.3	75.4
Heavy (>menses)*	95.1	47.4
Other		
Pain/cramps*	61.9	36.8
Fever	9.3	7.0
Physical restrictions*	11.4	3.5

*Difference between medical and surgical groups is significant at $p \leq 0.05$ as determined by χ^2 tests.

SYMPTOM DIARIES. An examination of symptom diaries (noting side effects experienced at any point during the study period) also provides insight into women's own perceptions of side effects. In all three countries, medical clients recorded significantly more side effects than did surgical clients on their diaries (Table 5). Indeed, gastrointestinal side effects, heavy bleeding, and pain or cramps were reported nearly twice as often (if not three, four, or even 10 times as often) in the diaries of medical clients than in those of their surgical counterparts. Fever, however, occurred infrequently (<10%) among all clients, regardless of the abortion method.

Although many women also noted abortion-related

physical restrictions,‡ occurrence of this symptom varied widely by site. In Cuba, fully one-third of medical (37.6%) and surgical clients (33.0%) reported physical restrictions at some point during their participation in the study, whereas, in India, only 11.4% of medical clients and 3.5% of surgical clients reported them. At all sites, however, more medical clients (China 26.5%, Cuba, 37.6%, India 11.4%) than surgical clients (China 12.6%, Cuba 33.0%, India 3.5%) reported physical restriction. Most medical abortion clients reporting physical restriction noted that it began 2 days after they received mifepristone (mean onset: China: day 2.7; Cuba: day 2.7; India: day 3.3), corresponding often to the day of misoprostol administration. For most surgical abortion clients, the exact onset of restriction varied across sites (mean onset: China: day 3.2; Cuba; day 1.1; India: day 7.5). Interestingly, despite fewer reports of physical restriction on their diaries, surgical abortion clients were generally restricted physically for a longer period of time by their abortion treatment than were medical abortion clients. For instance, in China, medical clients reported a mean of 4.1 days of physical restriction compared to 5.9 days for surgical clients. Similarly, in Cuba, medical abortion clients reported an average of 2.8 days of physical restriction, whereas surgical abortion clients noted being restricted for 3.5 days on average.

BLEEDING AND PAIN. Bleeding and pain are two of the most frequently reported side effects with both surgical and medical abortion. Indeed, in the case of medical abortion these side effects may drive a woman's decision to request a back-up intervention or a provider's decision to intervene.

On the whole, medical abortion clients reported significantly more blood loss than did surgical abortion clients, possibly because medical clients observe their blood loss, while surgical clients do not. Mean initial hemoglobin was identical for medical and surgical clients in China, and there was no significant difference in the mean changes in hemoglobin in the two groups (both rose slightly over the treatment period) or in the percentage of women who experienced a drop of >2 g/dL of hemoglobin (medical 6.0% vs surgical 5.2%). In both Cuba and India, the mean initial hemoglobin was slightly, but significantly, higher in the medical abortion group than in the surgical group. In both of these countries, mean changes in hemoglobin were also significantly greater in the medical group than in the surgical group (Cuba −0.57 g/dL vs −0.03 g/dL; India −0.29 g/dL vs −0.12 g/dL). In no site was the difference clinically meaningful: the percentage of women whose hemoglobin dropped >2 g/dL, a clinically meaningful indicator, was small and differed significantly between the two groups only in Cuba (medical 6.8% vs surgical 0.8%).[12]

‡ Physical restriction was defined as the inability to undertake one's daily routine activities, including (in this case) cooking and caring for children. This definition was adopted from the National Health Interview Survey.

Table 6. Pain relative to expectations, reported in exit interviews, by method and country (%)

	Medical	Surgical
China (n)	298	268
More*	14.4	26.5
Less*	71.1	48.9
As expected*	14.4	24.6
Cuba (n)	249	246
More*	24.5	16.7
Less*	48.6	60.6
As expected	26.9	22.8
India (n)	249	57
More	11.8	12.3
Less	65.9	59.6
As expected	22.4	28.1

*Difference between medical and surgical groups is significant at $p \leq 0.05$ as determined by χ^2 tests.

Most medical and surgical abortion clients reported less pain than expected (Table 6). Medical clients, however, more often characterized their pain as "less than expected" compared with their surgical counterparts in both China (medical 71.1% vs surgical 48.9%) and India (65.9% vs 59.6%). Nevertheless, "more pain than expected" was also noted by a fair number of women at all sites. About one quarter of Chinese surgical clients (26.5%) and Cuban medical clients (24.5%) did experience more pain than anticipated.

Concordance between actual experience and prior expectations of pain was closely connected to overall method satisfaction. Both surgical and medical clients experiencing more pain than expected were more likely to be less satisfied (medical $p < 0.001$, surgical $p < 0.001$) with their abortions. Conversely, those experiencing less pain than expected were more likely to be more satisfied (medical $p < 0.01$, surgical $p < 0.001$). When stratified by both method and by country, these associations were nearly always highly significant in all countries. Chinese medical and Indian surgical clients were the only exceptions to either pattern.

CLIENT WELL-BEING AND SATISFACTION. Despite the greater incidence of side effects noted by women and providers for the medical abortion clients, general assessments of well-being reported at exit interviews

Table 7. Client well-being and satisfaction at exit interview, by method and country (%)

	Medical	Surgical
Client well-being		
China (n)	299	268
Very well/excellent	41.8	34.0
Fair/comfortable/OK	52.2	59.3
Weak/not well	2.7	4.9
Worried but not sick	0.7	1.5
Other	2.7	0.4
Cuba (n)	249	256
Very well/excellent	73.1	69.9
Fair/comfortabe/OK	23.3	27.6
Weak/not well	1.6	1.6
Worried but not sick	1.2	0.0
Other	0.8	0.8
India (n)	249	57
Very well/excellent	59.8	63.2
Fair/comfortable/OK	32.9	28.1
Weak/not well	6.4	7.0
Worried but not sick	0.8	0.0
Other	0.0	1.8
Client satisfaction		
China (n)	299	268
Highly satisfied	42.8	23.1
Satisfied	51.5	72.8
Not satisfied	5.7	4.1
Cuba (n)	249	246
Highly satisfied	59.0	39.4
Satisfied	24.5	54.1
Not satisfied	16.5	6.5
India (n)	248	57
Highly satisfied	68.5	54.4
Satisfied	26.7	45.6
Not satisfied	4.8	0.0

Note: There were no significant differences between the medical and surgical groups.

were similar between women in the two treatment groups at each site (Table 7). Regardless of the method of abortion, the vast majority of women in Cuba (medical 73.1% vs surgical 69.9%) and in India (59.8% vs 69.9%) reported feeling "very well or excellent" at their exit interviews. In China, whereas slightly fewer women (41.8% vs 34.0%) than in the other two countries were as positive, most (52.2% vs 59.3%) noted that they felt "fair, comfortable, or okay." Very few women in all three countries reported that they were "worried, but not sick." More surgical abortion clients in China (2.7% vs 4.9%) and India (6.4% vs 7.0%), however, said they were "weak, not well." Similarly, nearly all women were "highly satisfied" or "satisfied" with their abortions, regardless of the method (Table 7). Medical abortion clients, however, were more often "highly satisfied" than their surgical counterparts (China 42.8% vs 23.1%, Cuba 59.0% vs 39.4%, India 68.5% vs 54.4%).

Discussion

To compare medical and surgical abortions, the intended effects (or "symptoms") of mifepristone and misoprostol need to be separated from the true "side effects" of the treatment. Just as topical anesthesia creates an intended effect of numbness to alleviate pain, the mifepristone-misoprostol regimen causes bleeding and abdominal cramping to provoke early abortion. Considering these features as "symptoms" rather than "side effects" (as is traditionally done) can guide the development of counseling and training materials.

Medical abortion clients in each site were more likely than surgical clients to report profuse and prolonged bleeding. Medical abortion clients observe more bleeding than do surgical clients, although the differences in blood loss are seldom clinically significant. Anticipating the bleeding that is normal for the method and distinguishing it from excessive bleeding that requires medical intervention will be an important aspect of providing medical abortion successfully. Well-developed counseling materials can help women anticipate the amount of bleeding that is normally expected and judge correctly when the bleeding is enough to warrant a clinic visit. Provider training will also help caution against over-diagnosing excessive bleeding to avoid unnecessary surgical procedures.

Medical abortion clients also reported substantial pain during their abortions. Pain that is correctly anticipated, however, can be largely managed with analgesics. Indeed, several medical abortion protocols[14] have adopted such a practice.§ As acceptability of the methods was influenced by expectations about pain, however, women will also need counseling materials to help them form realistic expectations about pain. Women should know what to expect so they can judge whether a method is right for them.

Despite the predominance of side effects among medical abortion clients, reports of well-being at the end of the study did not differ by method, and both methods proved highly acceptable at all sites. Reports of side effects among providers of medical abortion may decrease, however, as they become more experienced with the method and are better able to inform women about what to expect. Indeed, providers in the United States noted that their assessments of bleeding after medical abortion decreased as they gained confidence in the method.[15] Additionally, as common lore about the method increases among users and helps to draw a patient population well suited for the

§ Routine distribution of Tylenol® with codeine is being used in an ongoing medical abortion study in Tunisia.

regimen, reports by women of side effects are likely to decrease, as well.

Country differences also appear to influence women's reports and characterization of their side effects. Both Cuban medical and surgical clients, for instance, were particularly sensitive to pain, reporting far more cramping, pain, or physical restriction at all stages of their abortions than were their Chinese and Indian counterparts. Cuban women also stated more often that their pain exceeded their expectations. Similarly, regardless of the method of abortion, Chinese participants had more gastrointestinal side effects before and early on in their abortion process or procedure. Providers, too, seemed to differ in their assessments of side effects. For example, Indian providers noted more bleeding during the 4-h postmisoprostol observation period and characterized more women as bleeding heavily when they departed from the clinic after this period. These patterns suggest cultural variations in both definitions and perceptions of side effects, and warn against simple comparisons of side effects by various study regimens when the trials have been conducted in different cultures.

Finally, the present study results show that mifepristone itself appears to have very few side effects. If so, and if medical considerations are paramount, must its administration be strictly supervised, as has been the case in Europe? Misoprostol clearly produces more significant physical events; yet, a recent study allowing women to self-administer their prostaglandin at home demonstrates that medical supervision is not necessary even after misoprostol administration if counseling, expectations, and instructions on when to seek help are appropriate.[14]

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ORIGINAL RESEARCH ARTICLE

ELSEVIER

Determination of RU486 (Mifepristone) in Blood by Radioreceptorassay; A Pharmacokinetic Study

Imre Földesi,* George Falkay,† and László Kovács

A human progesterone receptorassay has been developed for the measurement of the biologically active molecular fraction of RU486 (RU486 binding equivalent) for studying its pharmacokinetic properties. Thirty-nine healthy pregnant volunteers with amenorrhoea of 49 days or less receiving a single oral dose of 200 mg, 400 mg or 600 mg RU486 orally in a single dose were involved in this study. Blood samples were collected within 48 hours for the analysis. It was found that the pharmacokinetics of the RU486 binding equivalent followed an open two-compartment model. The dose was rapidly absorbed and peak serum concentrations were measured within 1–2 hours after ingestion of the drug. The distribution was also rapid, but the elimination was slow, the elimination half-life ranging between 83 and 90 hours. Significant differences were found between the peak plasma values for the 200 mg and 600 mg doses ($p < 0.05$) and between the AUCs for the 200 mg and 600 mg doses ($p < 0.01$) and the 400 mg and 600 mg doses ($p < 0.05$). It can be concluded that this newly developed radioreceptorassay satisfies the requirements of radioligand binding techniques and can be used to determine the serum levels of RU486 and its metabolites, which are able to bind to human myometrial progesterone receptors. The pharmacokinetics for the RU486 binding equivalent is similar to that for RU486, with the exception of very slow elimination, which may originate from the measurement of the biologically active metabolites together with the parent compound. CONTRACEPTION 1996;54:27–32

KEY WORDS: mifepristone, pharmacokinetics, radioreceptorassay, RU486

Introduction

RU486 (mifepristone) is a synthetic 19-norsteroid that exhibits a great affinity for the progesterone and glucocorticoid receptors.[1,2] The dimethylaminophenyl side-chain on carbon 11 is important for the antiprogestogenic action of RU486.[3] Clinical trials worldwide have demonstrated its effectiveness in terminating unwanted pregnancy early in the first trimester.[4–7] The efficacy of this effect can be increased by combination with prostaglandins.[8]

After oral administration, the compound undergoes a relatively rapid metabolism (first pass effect). This occurs in three ways: mono- and didemethylation of the p-dimethylaminophenyl side-chain (RU 42633 and RU 42848, respectively) and hydroxylation of the propynyl group (RU 42698).[1] All of these metabolites retain antiprogestogenic and antiglucocorticoid activity in rats.[1] Relative binding affinity studies have demonstrated that the major metabolites of RU486 can also bind to the myometrial cytosolic progesterone receptor.[9] The plasma levels of the most potent RU486 metabolite (RU 42633) were higher than those of the parent compound.[9,10] These findings indicated that the RU486 metabolites may contribute to the overall effects of the drug in spite of their affinities being less than that of the parent compound.

Radioimmunoassay,[11–13] HPLC[10,13] and rat receptorassay[14] have already been developed for measurement of the serum concentrations of RU486 and its derivatives. We have developed a simple and sensitive radioreceptorassay (RRA) for the determination of RU486 and its biologically active metabolites in the serum. The interesting feature of this assay is the use of a human myometrial cytosolic progesterone receptor preparation as binding protein which is one of the main target tissues of antiprogestogens. The assay is based on the competitive replacement of [3H]ORG-2058 by the bioactive molecular fraction of RU486 extracted from the serum. The results were expressed as the RU486 binding equivalent, which involved all the RU486 derivatives able to bind to the human progesterone receptor. We studied the pharmacokinetics

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of the RU486 binding equivalent on the basis of serum concentrations measured by RRA.

Materials and Methods

Chemicals

RU486 [17β-hydroxy-11β-(p-dimethylaminophenyl)-17α-(1-propynyl)estra-4,9-dien-3-one] was kindly donated by Roussel-Uclaf Research Centre (Romainville, France). Diethyl ether and 5α-dihydrotestosterone (17β-hydroxy-5α-androstan-3-one) were purchased from Merck (Darmstadt, Germany). ORG-2058 was obtained from Organon (Oss, The Netherlands). Hydrocortisone (11β,17α,21-trihydroxy-pregn-4-ene-3,20-dione), tris-hydroxymethyl-amino-methane, magnesium chloride, EDTA, glycerol and Na-molybdate were from Sigma (St. Louis, Missouri, USA). [3H]ORG-2058 (49 Ci/mmol) and [3H]progesterone (65 Ci/mmol) were obtained from the Radiochemical Centre (Amersham, U.K.).

Subjects

This study was approved by the local Ethics Committee of the University. The women were examined and treated according to a WHO project protocol: 39 healthy pregnant volunteers, aged between 18 and 35 years, without detectable liver, renal, cardiovascular and endocrine disease, were involved in this study. They had normal menstrual cycles (25–35 days) for at least three months prior to conception and none had taken any steroid-containing drugs during this time. They had been amenorrhoeic up to 49 days with an ultrasonographically confirmed normal intrauterine pregnancy. Thirty-one women were randomized into three groups, receiving 200 mg (n = 9), 400 mg (n = 10) or 600 mg (n = 12) of RU486 orally in a single dose. Blood samples were collected at 0, 0.5, 1, 2, 4, 8, 12, 24, 36 and 48 hours after the administration of the drug. To study the total disappearance of the RU486, 8 pregnant women received 600 mg of RU486 and blood samples were taken at 0, 1, 2, 3, 7, 14 and 42 days after the intake of the RU486 pills. The serum samples were stored at -20°C until analyzed.

Preparation of Cytosolic Progesterone Receptors

Human uteri were obtained from women (aged 45–55 years) undergoing hysterectomy. Non-myomatous uterine tissues were homogenized in an assay buffer (20 mM Tris, 3 mM $MgCl_2$, 10 mM sodium molybdate, 1 mM EDTA, 10% glycerol, 0.01% sodium azide, pH 7.4) with an Ultra Turrax tissue homogenizer (Janke & Kunkel, Staufen, Germany) at 4°C. Cytosol samples were prepared by high-speed centrifugation for 1 hour at 40,000 g. The supernatant was collected and stored in aliquots in liquid nitrogen. It could be used up to 30 days without a detectable decrease in the progesterone receptors. The final protein content was measured by colorimetry[15] and ranged between 5 and 6 mg/ml.

Serum Extraction

One hundred µl serum samples were extracted twice with 3 ml of diethyl ether. The combined ether fractions were evaporated to dryness in a water bath and the residue was dissolved in 1.5 ml of assay buffer.

RRA of RU486 Binding Equivalent

Aliquots of cytosol (100 µl) were incubated with 5 nM [3H]ORG-2058 radioligand and RU486 standards (0–125 pmol/tube) or serum extracts in assay buffer containing 10^{-6} M 5α-dihydrotestosterone and 10^{-6} M cortisol to block sex hormone-binding globulin and corticosteroid receptors, respectively. The non-specific binding was determined in the presence of 1 µM ORG-2058 and was subtracted from each sample. After an overnight incubation at 4°C, 250 µl of dextran-coated charcoal was used for the separation of bound from free fractions. After incubation for 30 min at 4°C, the tubes were centrifuged for 10 min at 3000 g. The supernatants were transferred into counting vials and 5 ml of liquid scintillation fluid was added to each vial. The radioactivity of the bound fraction of the tritiated ligand was measured in an LKB Rackbeta 1211 liquid scintillation counter (Wallac, Turku, Finland). The results were calculated by means of the WHO Immunoassay Program (Version A5.2 by P.R. Edwards) and the amount of RU486 and its biologically active metabolites which displaced [3H]ORG-2058 from the progesterone receptor was expressed as the RU486 binding equivalent.

Reliability Criteria

The reliability of the RRA was examined according to Cekan.[16] The precision (reproducibility) of the developed RRA was determined by examination of 3 samples at the low, medium and high levels of RU486. The samples were measured 10 times in a single assay and in 10 different assays to calculate the intra- and interassay CV%, respectively. The average intra-assay CV% was 9.4% and the interassay CV% was 12.5%.

The validity of the RRA was assessed in a parallelism test. Serum samples containing high levels of RU486 were diluted with human female sera collected from the first trimester of their pregnancy (6–8 weeks of gestation) and analysed by RRA. Comparison of the dose-response relationship of the standards

and the diluted samples revealed parallelism between them at the 95% confidence level.

Influence of Endogenous Progesterone on RRA
The endogenous progesterone levels of the samples were also measured by RIA (WHO Matched Reagent Programme) together with RU486 determination by RRA. No correlation was found between them ($r = 0.125$), which indicates that the serum progesterone did not disturb the determination of the RU486 binding equivalent by RRA.

Extractability
To determine the efficiency of the extraction and recovery, human serum pools containing a known amount of RU486 at the low, medium and high levels were extracted and then measured by RRA. A very close correlation was found between the added and measured RU486 ($r = 0.98$). The recovery of the ether extraction ranged from 95 to 107%.

Pharmacokinetic Parameters of RU486 Binding Equivalent
An open two-compartment oral model was used for characterization of the pharmacokinetics of the RU486 binding equivalent. The following pharmacokinetic parameters were calculated from each individual patient by the iterative method, using the MedUSA computer programme (Medical Usage of Scientific Algorithms, Version 1.6, P. Várkonyi, 1990): absorption, distribution and elimination rate constants (k_a, α and β, respectively) and half-lives ($t_{1/2}k_a$, $t_{1/2}\alpha$ and $t_{1/2}\beta$, respectively), time to peak (t_{max}), peak levels (C_{max}) and areas under the concentration-time curves obtained by the trapezoid rule (AUC).

Statistical Analysis
Statistical comparison among the parameter groups was performed by one-way analysis of variance (ANOVA). Results were considered significant when $p < 0.05$. All calculations were done by SPSS statistical software (SPSS 6.0).

Results
Figure 1 depicts a typical calibration curve from the developed RRA. Each point represents a mean of triplicate determination. The sensitivity of the standard curve was 8.7 pmol/tube and the optimal measuring range was 10–120 pmol/tube.

Plasma concentrations of the RU486 binding equivalent measured within 48 hours after oral ingestion of 200 mg, 400 mg or 600 mg of RU486 are depicted in Figure 2. This shows that rapid absorption and distribution were followed by a relatively slow elimination, which demonstrates that the RU486 binding equivalent was still present in micromolar concentrations in the plasma at 48 hours. There was a marked between-subject variation in the plasma concentrations after the same RU486 dose. Figure 3 illustrates the total disappearance of the RU486 binding equivalent after the intake of a single oral dose of 600 mg RU486. The RU486 binding equivalent was found to be present in a measurable concentration in

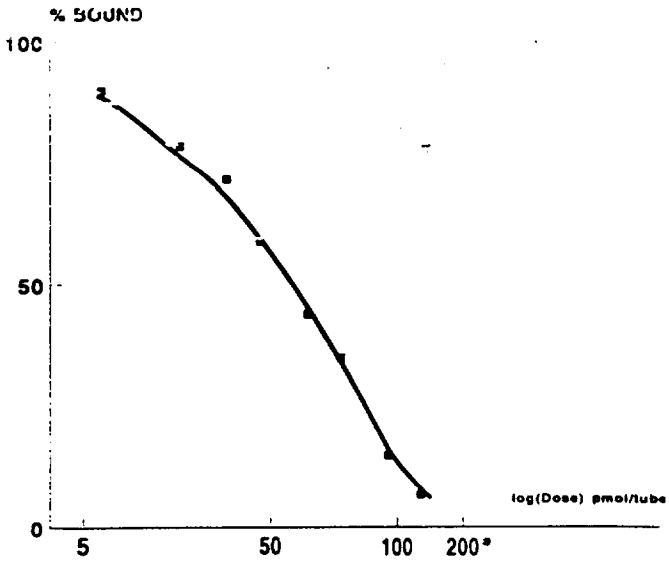

Figure 1. A representative calibration curve from the RU486 binding equivalent radioreceptorassay. Each point represents the mean of a triplicate determination.

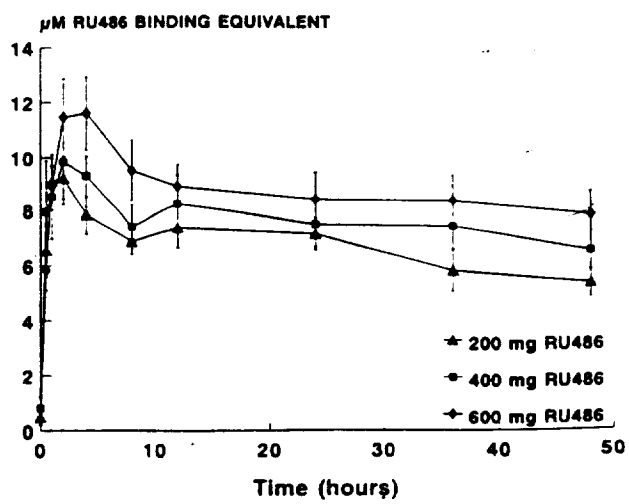

Figure 2. Serum concentrations of the RU486 binding equivalent (mean ± S.E.M.) measured within 48 hours by radioreceptorassay after a single oral dose of 200, 400 or 600 mg of RU486.

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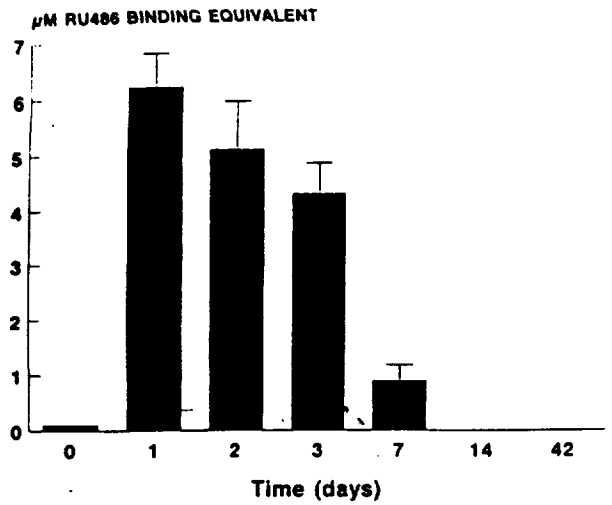

Figure 3. Serum concentrations of the RU486 binding equivalent (mean ± S.E.M.) within 42 days after a single oral intake of 600 mg of RU486, as determined by radioreceptor-assay.

the serum on the 7th day after the administration, but decreased below the RRA detection limit between the 7th and 14th days.

Pharmacokinetic parameters of the RU486 binding equivalent are listed in Table 1. The rapid absorption was reflected by the small values of k_a and the rapid absorption half-life at all three doses. The absorption was followed by a rapid distribution from the central to the peripheral compartment. In contrast, the elimination of the RU486 binding equivalent from the circulation was very slow, which accounts for the long elimination half-life ($t_{1/2}\beta$ ranged between 83 and 90 hours) and the elimination rate constants. The inter-subject variability in the pharmacokinetic parameters was also very large.

The dose-related pharmacokinetic parameters are shown in Table 2. Times to peak, peak values and areas under the curves were calculated. The concentration of the RU486 binding equivalent reached its maximum within 2 hours, demonstrating a rapid absorption. The C_{max} values were 9.30, 10.65 and 12.30 μmol/l after the oral administration of 200 mg, 400 mg and 600 mg of RU486, respectively. There was a significant increase in C_{max} on passing from the 200 mg to the 600 mg oral dose ($p < 0.05$). From the aspect of the bioavailability as assessed via the AUC, significant differences were found between the 200 mg and 600 mg doses ($p < 0.01$) and the 400 mg and 600 mg doses ($p < 0.05$), but these elevations were not directly proportional to the increase of the dose.

Table 2. Dose-related pharmacokinetic parameters of the RU486 equivalent (time to peak, peak level and AUC) after a single oral dose of 200, 400 and 600 mg RU486; values are means ± SD

RU486 Doses	t_{max} (h)	C_{max} (μmol/l)	AUC (h × μmol/l)
200 mg (n = 9)	1.71 ± 0.54	9.30 ± 2.22	930.4 ± 270.5
400 mg (n = 10)	2.05 ± 0.98	10.65 ± 3.37	978.1 ± 227.0
600 mg (n = 12)	1.73 ± 0.71	12.30 ± 3.52*	1272.0 ± 298.3†‡

*$p < 0.05$, compared with 200 mg dose.
†$p < 0.01$, compared with 200 mg dose.
‡$p < 0.05$, compared with 400 mg dose.

Discussion

A number of studies on the pharmacology of RU486 have already been published.[1,17–21] It is well known that RU486 is rapidly metabolized in the splanchnic circulation after oral administration.[1] In humans, micromolar concentrations of monodemethylated (RU 42633), didemethylated (RU 42848) and hydroxylated (RU 42698) metabolites were observed within 1 hour after the oral intake of RU486.[9] The serum concentrations of the metabolites (especially RU 42633) attained their peak levels 1–2 hours after the administration and remained higher than that of the parent compound for at least 72 hours.[9] The affinities of the metabolites for the human uterine progesterone receptor are less than that of RU486, but still considerable.[1,9] RU 42633 and RU 42698 can interrupt pregnancy in rats.[1] Although the biological effects of the major metabolites of RU486 have not been evaluated directly in humans, they may contribute to the pharmacological action of RU486 because of their high concentrations in the serum.

We have developed a sensitive RRA for the deter-

Table 1. Calculated pharmacokinetic parameters of the RU486 binding equivalent (absorption, distribution and elimination) after a single oral dose of 200, 400 and 600 mg of RU486; values are means ± SD

RU486 Doses	k_a (1/h)	$t_{1/2}(k_a)$ (h)	α (1/h)	$t_{1/2}(\alpha)$ (h)	$\beta(\times 10^{-3})$ (1/h)	$t_{1/2}(\beta)$ (h)
200 mg (n = 9)	1.60 ± 0.73	0.54 ± 0.28	0.83 ± 0.49	1.12 ± 0.62	8.36 ± 2.69	90.8 ± 26.2
400 mg (n = 10)	1.77 ± 0.79	0.45 ± 0.17	1.04 ± 0.53	0.84 ± 0.41	8.28 ± 1.98	84.5 ± 21.8
600 mg (n = 12)	1.65 ± 0.65	0.49 ± 0.34	1.01 ± 0.69	0.81 ± 0.54	8.91 ± 2.77	83.6 ± 21.2

mination of RU486 and its biologically active metabolites. The human myometrial cytosolic progesterone receptor was used as binding protein, which is the target receptor for RU486 binding. A similar method has already been developed by Kawai et al.,[14] who used a rat uterine progesterone radioreceptorassay. The species specificity is very important because the rat progesterone receptor binds RU486 with higher affinity than does the human receptor. On the other hand, the hamster progesterone receptor does not recognize RU486.[22,23] There is a difference in the hormone binding domains of the hamster and human progesterone receptors. The amino acid glycine at position 722 is essential for RU486 binding. The hamster progesterone receptor contains cysteine instead of glycine at this position.[24] The chicken progesterone receptor has the same property.[25]

The assay is based on the competitive replacement of [3H]ORG-2058 by the biologically active molecular fraction of RU486. The reliability criteria of this newly developed RRA satisfy the requirements of radioligand binding techniques. Our measured data were used to investigate the pharmacokinetics of the RU486 binding equivalent after a single oral intake of three different doses (200 mg, 400 mg, 600 mg) of RU486. The results from previous single-dose studies were compared with our data. We found a wide intersubject variability in the serum concentrations and in the pharmacokinetic parameters. These findings agree with the previously reported data.[21] An open two-compartment oral model was applied to describe the pharmacokinetics of the RU486 binding equivalent. All studies revealed that the time required (t_{max}) to achieve C_{max} was low, indicating rapid absorption.[12,17,20,21] This procedure was followed by a rapid distribution and a relatively slow elimination. As demonstrated in Table 1, the mean half-life of absorption was less than 1 hour. T_{max} was also low, which confirmed the rapid absorption. The absorption was followed by a rapid distribution with a mean half-life of 0.81–1.12 hours, but the elimination process was very slow.

The time for disappearance of the RU486 binding equivalent from the circulation was very long. We found a measurable amount of the RU486 binding equivalent even on the 7th day after the intake of a single oral dose of 600 mg of RU486, but none was detected on the 14th and 42nd days. Thus, the total disappearance of 600 mg of RU486 took about 7–14 days. This finding is in good agreement with previously published data.[12,17,21] Consequently, $t_{1/2}\beta$ lay in the range 83–90 hours. This is a very long elimination as compared with earlier data based on the determination of RU486 by RIA and HPLC. The average elimination half-life of RU486 was from 24 to 35 hours,[10,17,20,21] and only Liu et al.[18] reported a longer $t_{1/2}\beta$ (about 53 hours) from a RIA determination. Lähteenmäki et al.[17] demonstrated that, after the ingestion of RU486 doses higher than 100 mg, there is a redistribution phase within 6–10 hours, after which the level of RU486 attains a plateau for 24–48 hours. It should be noted that we measured the serum concentrations of the RU486 binding equivalent only for 48 hours. Thus, terminal elimination half-lives calculated on the basis of this interval can only be accepted as approximate values. When the dose is higher than 50–100 mg, the specific high-affinity carrier protein of RU486, the human orosomucoid, becomes saturated and RU486 starts to enter the tissues.[17] Heikinheimo found that the in vitro RU486 accumulation in the adipose tissues was less than expected in the presence of α_1-acid-glycoprotein. He suggested that other mechanisms, e.g., the enterohepatic circulation, may also be responsible for the long elimination of RU486.[19]

The peak level and AUC are very important parameters in studies of the dose-related pharmacokinetics and bioavailability. We have found that the RU486 binding equivalent level was higher than that of RU486 as measured by HPLC; this was not surprising as the RRA measured the bioactive metabolites together with the parent compound. Significant increases were found in C_{max} on passing from the 200 mg dose to the 600 mg dose ($p < 0.05$), and in the AUC on passing from the 200 mg and 400 mg dose to the 600 mg dose ($p < 0.01$ and $p < 0.05$, respectively). However, these differences were not directly proportional to the increase of the dose.

Although the HPLC is more specific in studies on the pharmacokinetics and metabolism of RU486, this newly developed radioreceptorassay satisifies the requirements of radioligand binding techniques and can give comparable results with the HPLC and RIA. The RRA applying an appropriate standard, can also be used to determine the serum concentrations of other compounds (e.g., ZK98734, Schering AG., our unpublished data) able to bind to human myometrial progesterone receptors. The pharmacokinetics of the RU486 binding equivalent is similar to that for RU486. However, we found that the elimination half-lives were very long as compared with previously published data. This difference may be caused by the metabolites being measured together with RU486. It is important to stress that the human myometrial cytosolic progesterone receptor was used as binding protein, which is the target receptor for antiprogestins, and we determined only the biologically active derivatives. Our findings suggest that the pharmacological effect of RU486 is prolonged after a single oral dose. Further, the biologically active me-

tabolites of RU486 (especially the monodemethylated metabolite, whose concentrations were higher than those of RU486 in all tissues examined, e.g., human myometrium[19]) may play an important role in the mechanism of the antiprogesterone action of RU486.

Acknowledgments

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Mifepristone in combination with methotrexate for the medical treatment of tubal pregnancy: a randomized, controlled trial

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In the search for a more potent alternative to a single i.m. injection of methotrexate for ectopic pregnancy, a randomized trial was organized. The efficacy of a combination of methotrexate and mifepristone was compared with methotrexate alone in the treatment of unruptured tubal pregnancies. The diagnosis of an unruptured tubal pregnancy was confirmed laparoscopically in 50 patients during a 2 year period. Women were randomized to receive a single i.m. injection of 50 mg/m² methotrexate alone or a single dose of 600 mg oral mifepristone in combination with the same dose of methotrexate. Both treatment protocols were successful in achieving the resolution of unruptured ectopic pregnancy (18/25 in the methotrexate group and 22/25 in the combination group) following the initial intervention. A second injection was needed in four (16%) cases in the methotrexate group and in one (4%) case in the combination group. Overall, a complete resolution was achieved in 22/25 and 23/25 cases respectively. Unruptured ectopic pregnancy resolved faster in women given the combination of methotrexate and mifepristone compared to women given methotrexate only (P = 0.01). The effect of the methotrexate and mifepristone combination was more pronounced in women with higher human chorionic gonadotrophin concentrations.

Key words: methotrexate/mifepristone/tubal pregnancy

Introduction

The incidence of ectopic pregnancy has almost doubled in the Western world since the 1960s and ranges from 0.25–1% of all pregnancies (Stabile and Grudzinskas, 1994). High resolution ultrasound and readily available β-human chorionic gonadotrophin (HCG) assays have enabled the earlier diagnosis of ectopic pregnancy, which is often unruptured. Diagnosis of an unruptured ectopic pregnancy allows therapy to be either medical or surgical.

Outcomes of laparoscopic linear salpingostomy, which is arguably the most suitable surgical option for unruptured isthmic or ampullary gestations not larger than 4 cm, show that 95% of procedures are successful with a recurrence rate of 22% (Buster and Carson, 1995). Following laparoscopic surgery, postoperative complications such as bleeding, elevated HCG, or other persisting symptoms occur in up to 20% of cases. Clasen *et al.* (1997) suggested that a surgical approach by means of the laparoscope should remain the gold standard and should be optimized further, at least until more data are available to evaluate the true value of the non-surgical approach. Maymon and Shulman (1996) suggested that the first line treatment should be laparoscopic salpingostomy or ultrasound guided methotrexate injection and that further studies were needed to compare different conservative treatment options. It must be emphasized that laparoscopic surgery requires special instruments and expertise which may not always be readily available.

However, if the tubal ectopic pregnancy is not larger than 4 cm in diameter, a single i.m. dose (50 mg/m²) of methotrexate is effective in more than 90% of cases (Stovall and Ling, 1993). A second injection may be needed in 3% of cases. Corsan *et al.* (1995) reported mild, self-limited side-effects in 41% of women following i.m. methotrexate administration. Exacerbation of abdominal pain after treatment was noted by 59% of patients (Stovall and Ling, 1993). Tubal patency is often conserved and the recurrence rate is very low (12%) (Stovall and Ling, 1993). However, failed treatment may be life threatening and therefore further improvement is required if medical treatment is to become an accepted first line management of unruptured tubal pregnancy.

Mifepristone is the first clinically effective antiprogestagen. It is already being used to induce first trimester abortion particularly in conjunction with a prostaglandin (UK Multicentre Trial, 1990; Ulmann *et al.*, 1992), but has been unsuccessful in the treatment of ectopic pregnancies (Paris *et al.*, 1986a, b; Kenigsberg *et al.*, 1987; Levin *et al.*, 1990; Tulandi, 1992; El-Rafaey *et al.*, 1993). There is, however, evidence to suggest that mifepristone may have some beneficial effect in the treatment of ectopic pregnancy (Dietl, 1992). We hypothesized that whilst the effect of mifepristone on the development of an ectopic pregnancy may not be strong enough to cause resolution, it may still have a clinically important negative effect on the development of the ectopic trophoblast.

The purpose of this study was to test the hypothesis that a combination of methotrexate and mifepristone could provide a more potent alternative for the medical treatment of ectopic pregnancies. We conducted a prospective, randomized trial to compare the efficacy of 50 mg/m² i.m. methotrexate injection alone with a combination of 50 mg/m² methotrexate injection

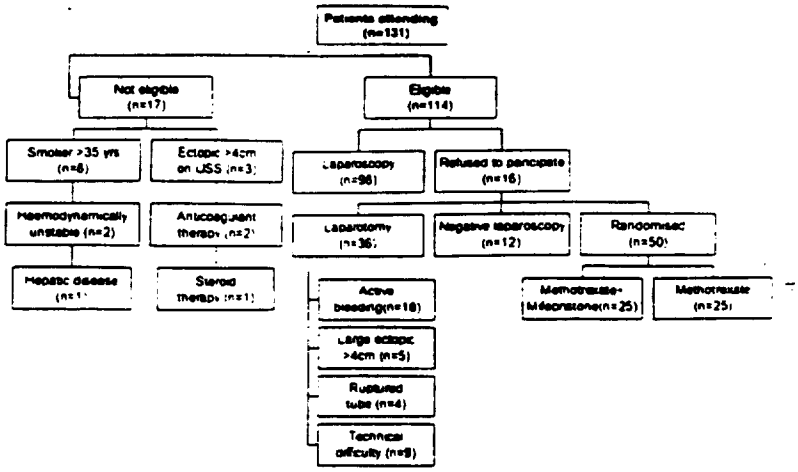

Figure 1. Summary of recruitment and treatment data. USS = ultrasonography.

Table I. Demographic details

	Methotrexate + mifepristone (n = 25)	Methotrexate only (n = 25)
Age (years)[a]	29 (4.4)	27 (3.7)
Parity[b]	1 (0–2)	1 (0–2)
Weight (kg)[b]	58 (47–72)	55 (45–69)
HCG at randomization (IU/l)[b]	497 (30–4200)	346 (52–12 700)
Abdominal pain (%)	22 (92)	21 (84)
Vaginal bleeding (%)	15 (63)	16 (64)
Peritoneal fluid on scan (%)	23 (96)	24 (96)

[a]Mean (SD).
[b]Median (range).
HCG = human chorionic gonadotrophin.

and 600 mg of oral mifepristone in the treatment of unruptured tubal ectopic pregnancy.

Materials and methods

Between April 1994 and April 1996, 131 women who presented to the Early Pregnancy Unit at Singleton Hospital, Swansea, UK, with suspected ectopic pregnancy had symptoms of abdominal pain only (n = 121), vaginal bleeding only (n = 10) and pain with bleeding (n = 87). All patients had a urine pregnancy test and a vaginal ultrasound scan examination. Haemodynamically unstable patients, patients with hepatic or renal dysfunction, ectopic pregnancies with a diameter greater than 4 cm on ultrasound scan, smokers over the age of 35 years, long term corticosteroid users, and patients with haemorrhagic disorders or who were on anticoagulant therapy were not invited to take part in the study (n = 17) (Figure 1). The remaining 114 patients were invited to take part in the trial. Ninety-eight patients agreed to take part and had a diagnostic laparoscopy to diagnose positively an unruptured tubal ectopic pregnancy. Women were counselled and an informed consent obtained for the medical treatment prior to laparoscopy.

Unruptured ectopic pregnancy with a diameter not larger then 4 cm was diagnosed in 50 cases (51%). Twelve (12%) women were diagnosed as not having an ectopic pregnancy. In 36 (37%) cases the operating surgeon decided against medical treatment due to a ruptured tube (n = 4), large ectopic >4 cm (n = 5), active bleeding from the fimbrial opening (n = 18) or technical difficulties due to adhesions and/or blood where visualization was limited (n = 9) and a laparatomy was carried out (Figure 1).

Following laparoscopic diagnosis of an unruptured tubal ectopic pregnancy and peritoneal lavage, a consecutively numbered envelope was opened and 50 women were randomly allocated to one of the treatment groups. A computer generated randomization sequence was used in the preparation of the envelopes. Women in the methotrexate only group received a single i.m. injection of 50 mg/m^2 body surface and women in the combination group received the same dose of methotrexate and a single dose of oral mifepristone (600 mg).

Following treatment women were allowed home within 24 h. They were reviewed on the 4th and 7th day on an out-patient basis. All women had blood HCG estimations, hepatic and renal function tests and full blood counts (red cells, white cells and platelets) on each visit. If the HCG concentrations dropped by more than 15% between days 4–7, the women were then reviewed weekly until the HCG concentrations were below 12 IU/l. If the decrease was less than 15% between days 4–7 then a second dose of methotrexate was administered. In these cases, blood HCG concentrations were estimated also on days 11 and 14. On each visit women were asked about side-effects and complaints. These were recorded on data sheets. Advice was given regarding the use of contraception to avoid pregnancy for 3 months following the methotrexate administration.

The first 25 women were offered hysterosalpingograms (HSG) to assess their tubal patency once the complete resolution of the ectopic pregnancy was achieved. Ten women out of the first 12 from the methotrexate only group and 10 out of the first 13 women from the combination group agreed to have a HSG following their first normal period. The mean time interval from treatment to HSG was 45 days (±8.4) in the methotrexate group and 36 days (±7.2) in the combination group.

The primary outcome measure was the complete resolution of ectopic pregnancy defined as HCG concentrations <12 IU/l following the initial treatment. The secondary outcome measures were the need for a second injection, resolution interval, need for laparotomy and conservation of tubal patency.

Results were analysed on an intention to treat basis. In the statistical analysis, non-parametric data were described as median (range) and parametric data as mean (±SD). Differences between the groups were analysed using Fisher's exact test for non-parametric and χ^2 test for parametric data. Continuous variables were analysed using the Mann–Whitney U test for non-parametric data. The Kaplan–Maier test was used for median resolution times. The stratified log-rank test was used to calculate the χ^2 for equivalence of resolution

Table II. Management and outcome

	Methotrexate + mifepristone (n = 25)	Methotrexate only (n = 25)	Odds ratio	95% CI
Initial intervention successful (%)	22 (88)	18 (72)	2.85	0.54–19.17
Methotrexate (second dose) (%)	1 (4)	4 (16)	0.22	0.004–2.51
Complete resolution (%)	23 (92)	22 (88)	1.57	0.16–20.28
Laparotomy (%)	2 (8)	3 (12)	0.64	0.05–6.19
Outpatient visits (number)[a]	3 (2–7)	4 (2–10)		0–2
Time to resolution (days)[a]	14 (7–35)	21 (7–63)		0–7
Nausea (%)	2 (8)	2 (8)		

[a]Median (range).
CI = confidence interval.
None of the differences between treatment groups was significant.

rates and the difference between the groups, taking into account the initial concentrations of HCG, success of the treatment and resolution rates. As this was a feasibility study, sample size was not based on pre-specified power calculations. The aim was to recruit all eligible women in a 24 month period.

Results

Twenty-five patients were randomized to receive methotrexate only and 25 patients to receive methotrexate and mifepristone. There were no differences between the groups regarding the age, parity, body weight and initial concentrations of HCG (Table I). A complete resolution of the ectopic pregnancy following the initial intervention was achieved in 22 cases (88%) of the methotrexate group and in 23 cases (92%) of the combination group. A second injection was needed in four cases (16%) in the methotrexate group, and in one case (4%) in the combination group. Similar rates were observed in the incidence of laparotomies: three (12%) and two (8%) respectively. The median resolution (HCG < 12 IU/l) time in the methotrexate group was 21 days (range 7–63) and 14 days (range 7–35) in the combination group (Table II).

There were three cases of laparotomy in the methotrexate group. One woman had a rise in serum HCG concentrations between days 4 and 7 and there was abdominal pain. A second injection of methotrexate was offered but the patient decided to withdraw from the trial and requested a laparotomy and salpingectomy. The operation confirmed an unruptured cornual pregnancy which was missed at the initial laparoscopy. In the second case the patient had severe abdominal pain on day 5 of the initial diagnosis. A vaginal ultrasound examination confirmed that the ectopic pregnancy had increased in size (>5 cm). A laparotomy and salpingectomy was carried out and an enlarged but unruptured tube was found. The third patient had falling HCG concentrations from 11478 IU/l to 162 IU/l within 7 weeks. However her symptoms of abdominal pain persisted. A laparotomy and salpingectomy was performed which confirmed a 6 cm long, unruptured Fallopian tube enlarged along its horizontal axis. It contained resolving products of conception.

In the combination group there were two laparotomies. In the first case abdominal discomfort persisted despite falling HCG concentrations. A laparotomy and salpingectomy 5 weeks after the initial treatment confirmed an unruptured ectopic pregnancy. In the second case a second methotrexate injection was administered on day 7 due to raised HCG concentrations. A satisfactory drop in HCG concentrations was achieved (from 4682 IU/l to 3884 IU/l, 17%) within a week. The patient was essentially asymptomatic. However, a clinical decision was then taken to perform a laparotomy and linear salpingostomy. All surgical interventions in our trial were due to doctors' bias as all patients were haemodynamically stable.

There were 45 women in whom tubal pregnancy had resolved successfully following the medical treatment: 22 in the methotrexate only group, 23 in the combination group. Using the stratified log–rank test, the overall χ^2 for the equivalence of the resolution rates was 6.91 (P = 0.01) confirming the significant difference between the treatment groups. Having divided the data from resolved pregnancies in both groups (n = 45) according to the median HCG on day 1 (423 IU/l), χ^2 for the equivalence of resolution rates between the groups for patients with a concentration of HCG over 423 IU/l on day 1 was 7.21 (P = 0.01) and for patients with a HCG concentration below 423 IU/l was 1 (P = 0.3), indicating significant difference of the resolution rates between two treatment arms in patients with higher concentrations of initial HCG (>423 IU/l).

Two women in each group reported mild nausea. The platelet and white cell count, hepatic and renal function tests stayed stable in all women. None of the patients had evidence of pancytopenia.

In all 20 women who had hysterosalpingograms the tubes in which the ectopic pregnancy was initially diagnosed were found to be patent.

Discussion

The combination of methotrexate with mifepristone appears to have had a stronger effect on the resolution of unruptured ectopic pregnancies compared to methotrexate alone, although the numbers were relatively small and the data are limited. The success rates for treatment arms were similar, however, median administration to resolution times were shorter and a second injection or laparatomy was less likely to be needed in the combination group. Our results suggest that mifepristone in combination with methotrexate has a more pronounced effect on the resolution of tubal pregnancy when the initial HCG concentrations are high (>423 IU/l).

Our overall success rate of medical treatment was 90% which is comparable to previously reported trials using the same dose of i.m. methotrexate (Stovall and Ling, 1993; Glock *et al.*, 1994; Gross *et al.*, 1995). We analysed our data on an intention to treat basis and included all women randomized for the trial. One woman in the methotrexate only group withdrew from the trial and opted for laparotomy on day 7 when she was asymptomatic. Another laparotomy, from the combination group, was a violation of the trial protocol as the patient was asymptomatic and HCG concentrations were falling.

In our trial the diagnosis of an unruptured ectopic pregnancy was made laparoscopically. Laparoscopy is the gold standard diagnostic test in women with suspected ectopic pregnancy and it can be carried out by on-call gynaecologists throughout the U.K. Laparoscopic assessment and peritoneal lavage enabled women with fluid in the pouch of Douglas, who would otherwise be unsuitable, to be considered for medical treatment.

Laparotomies in our trial were carried out semi-electively, i.e. none of the patients had haemodynamic complications or collapse. We suggest that emergencies are likely to be avoided if laparoscopic diagnosis is used.

We agree that efforts should be focused on the non-surgical diagnosis of intact ectopic pregnancy. However, current protocols include serial HCG measurements and endometrial biopsies which may cause considerable delay before the diagnosis is made (Vermesh, 1989; Ling and Stovall, 1993). Furthermore, proposed diagnostic protocols suggest that the presence of fluid in the pouch of Douglas on transvaginal scan should be an indication for diagnostic laparoscopy to exclude tubal rupture. It is worth noting that out of 50 women with unruptured ectopic pregnancy in our trial only three did not have fluid in the pouch of Douglas.

In instances where the diagnosis remains uncertain, laparoscopy will remain invaluable. In such cases women should be given the choice of medical treatment. We believe that the combination of methotrexate with mifepristone offers a better alternative to single dose methotrexate. If better conservation of the reproductive function and lower complication rates are confirmed by larger studies, medical treatment even after a diagnostic laparoscopy may be a viable option to laparoscopic salpingostomy.

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The effect of various doses of mifepristone on endometrial leukaemia inhibitory factor expression in the midluteal phase—an immunohistochemical study

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Leukaemia inhibitory factor (LIF) is a cytokine which plays an obligatory role in mouse blastocyst implantation. In human endometrium, LIF expression is significantly increased in the mid-luteal phase indicating that LIF may also play an important role in the human. We have previously shown that a single dose of 200 mg of mifepristone immediately post-ovulation is an effective contraceptive method, probably due to inhibition of endometrial development and function. The purpose of this study was to investigate the effect of various doses of mifepristone on endometrial LIF expression. A total of 22 fertile, regularly-menstruating women were studied during control and treatment cycles. The subjects were divided into four groups: group I received a single dose of 200 mg of mifepristone on cycle day LH + 2 (n = 7). The subjects in groups II and III were treated with either 5 mg (n = 5) or 2.5 mg (n = 5) once a week for 2 months. Group IV subjects received 0.5 mg per day (n = 5) of mifepristone for 3 months. LIF was measured immunohistochemically in endometrial tissue specimens taken on the corresponding day (cycle day LH + 6 to LH + 8) in hormonally-characterized control and treatment cycles. LIF immunostaining was observed in all controls and located to the cytoplasm of the luminal and glandular epithelial cells and stromal cells. In the treatment cycles the staining of luminal epithelium and stroma was similar to controls, while the glandular staining was reduced in all treatment groups. This study reveals that early luteal phase treatment as well as intermittent or daily low dose treatment with mifepristone reduces endometrial glandular LIF expression at the expected time of implantation. The results further support the contraceptive potential of mifepristone in low doses.

Key words: antiprogestin/endometrial receptivity/implantation/LIF

Introduction

Implantation is a sequence of events whereby the blastocyst becomes attached to the uterine wall. Mammalian implantation involves an extensive interaction between the embryo and endometrium. Although the regulation of implantation is not clearly understood, it is well known that progesterone is essential for the development of a receptive endometrium. Recent studies suggest a critical role for autocrine/paracrine factors such as cytokines in this process, which may be the effectors of the steroid hormones. Cytokines are not only hormonally regulated but also regulate each other in a cascade process. Leukaemia inhibitory factor (LIF) is a cytokine which plays an obligatory role in mouse blastocyst implantation. The role of uterine expression of LIF in implantation was demonstrated in transgenic mice homozygous for LIF deficiency. Implantation and normal development of these embryos occurred when transferred to wild type recipients or when exogenous LIF was given to the LIF deficient female (Stewart *et al.*, 1992). Wild type blastocysts could not implant when transferred to homozygous mice. Human endometrium has been found to be an active site for cytokine growth factor production and action (Giudice, 1994; Tabibzadeh, 1995; Simón *et al.*, 1996). Expression of endometrial LIF mRNA and protein changes during the menstrual cycle and is significantly increased in the midluteal phase.(Charnock-Jones *et al.*, 1994). The high level of endometrial LIF secretion at a time when implantation normally occurs indicates that LIF may also play an important role in human endometrial receptivity and implantation.

The development of antiprogestins offers a way to study progesterone-dependent mechanisms involved in the process of successful implantation such as the development of a receptive endometrium. Treatment with 200 mg of mifepristone or 400 mg of onapristone on cycle day LH + 2 did not significantly affect the pattern of LIF immunostaining in endometrial biopsies taken on cycle day LH + 4. However, reduced glandular staining was evident on cycle day LH + 6 (Cameron *et al.*, 1996). Administration of an antiprogestin immediately post ovulation is an effective contraceptive method (Gemzell Danielsson *et al.*, 1993). The contraceptive effect seems to be primarily due to inhibition of endometrial development and function (Swahn *et al.*, 1990; Mäentausta *et al.*, 1993; Gemzell Danielsson and Hamberg, 1994; Gemzell Danielsson *et al.*, 1994). It was recently shown that low intermittent or daily doses of mifepristone disturb endometrial maturation and secretory activity without inhibiting ovulation and the normal rhythm of the menstrual cycle (Gemzell Danielsson *et al.*, 1996, 1997). Whether the effect of low doses of mifepristone is sufficient to prevent implantation remains to be established.

The purpose of this study was to investigate the effect of various doses of mifepristone on the endometrial expression of LIF as a potential contraceptive method.

The study was approved by the ethics committee of the Karolinska Hospital. Informed consent was obtained from each subject before inclusion in the study.

Materials and methods

A total of 22 healthy women, 22–40 years of age, with regular menstrual cycles (25–35 days) volunteered for the study. None of the women had used steroidal contraceptives or an intrauterine device for a minimum of 3 months prior to the study. Gynaecological examination was performed on admission. The subjects who were not sterilized were advised to use barrier methods for contraception during the study and all subjects were asked to keep daily records on any side-effects and bleeding.

The study included one control and one to three treatment cycles. The subjects were randomly assigned to four groups. Group I received a single dose of 200 mg of mifepristone on cycle day luteinizing hormone (LH) +2 (n = 7). The subjects in groups II and III were treated with either 5 mg (n = 5) or 2.5 mg (n = 5) of mifepristone once a week for 2 months starting on cycle day 2. The subjects in group IV received 0.5 mg of mifepristone as a daily dose for 3 months (n = 5). In all treatment groups, endometrial biopsies were taken on the corresponding day (cycle day LH + 6 to LH + 8) of control and treatment cycles. The biopsies were obtained from the uterine fundus using a Randall curette without prior cervical dilatation or anaesthesia. All specimens were frozen immediately and stored in liquid nitrogen until analysed.

Hormone assessment

During the control and treatment cycles, serum and urine samples were collected. The samples were analysed for pregnanediol glucuronide, oestrone glucuronide and luteinizing hormone (LH) using radioimmunoassay (Cekan *et al.*, 1986). The hormone concentrations in urine were expressed per gram creatinine (Metcalf and Hunt, 1976). For creatinine analysis a commercial kit (Sigma Diagnostics, St Louis, MO, USA; Procedure no. 555) was used. In addition, all subjects determined the LH peak in urine samples collected twice daily from approximately cycle day 10 to LH + 2 by using a rapid self-test (Clearplan, Searle Unipath Ltd, Bedford, UK).

Immunohistochemical analyses

The endometrial specimens were mounted in an embedding medium (OCT Compound; Miles Inc, Elkhart, IN, USA) and sectioned to 8–10 μm. The sections were placed on glass slides and air-dried for 15–20 min before 10 min fixation in acetone. The slides were washed three times with phosphate-buffered saline (PBS) for 2 min each, incubated in H_2O_2 (0.3% in MEOH) for 5 min to block endogenous peroxidase activity and then washed 3×2 min in PBS prior to incubation with normal horse serum (1.5% for 30 min). Polyclonal LIF antibody (R&D Systems, Minneapolis, MO, USA) diluted 1:80 was supplied and incubated overnight. The slides were then washed three times in PBS for 2 min. Specific binding of the primary antibody was detected using a complex of biotinylated horse anti-goat immunoglobulin (vector diluted 1:200 for 30 min). The slides were then washed in PBS prior to incubation for 30 min with AB Complex (Vectastain ABC kit, Vector Laboratories Inc., Burlingham, CA, USA; prepared according to the manufacturer's instructions). After PBS washing the peroxidase substrate solution *p*-amino-ethyl carbazole (ACE; Sigma) was added. When incubated for 5 min the sections were rinsed with tap water, counterstained with haematoxylin (Kebo, Stockholm, Sweden) 10%, 3 min and mounted with coverslips. Control incubations induded deletion of the primary antibody. The occurrence of specific staining for LIF was characterized as absent (–; 0%), weak (+; 1–30%), moderate (++; 31–60%), or strong (+++; 61–100%). A control specimen was always included with the treatment biopsies as a positive control.

Morphometric analyses

One part of the biopsy material was immediately fixed in Bouin's solution and used for light microscopic examination (×400) after embedding in paraffin and stained with haematoxylin. Morphometric analyses were performed measuring number of glands per mm^2, number of glandular and stromal mitoses per 1000 glandular or stromal cells respectively, glandular diameter (μm), glandular epithelial height (μm), cells with basal vacuolization per 1000 glandular cells as well as the number of pseudostratified cells and the degree of stromal oedema (Johannisson *et al.*, 1987). Microscopic evaluation of the samples was performed at the end of the study by one person, who was unaware of the precise cycle day and whether the biopsy was taken in the control or treatment cycle.

Statistical analysis

Differences in morphometric data were evaluated by using the two-tailed Wilcoxon signed-rank test. $P < 0.05$ was considered to be statistically significant.

Results

All control and treatment cycles were ovulatory with an LH peak. Urinary concentrations of oestrone glucuronide and pregnanediol glucuronide, LH and serum concentration of cortisol were not significantly affected by the administration of mifepristone (data not shown). However, following treatment with 5 and 2.5 mg of mifepristone once a week, ovulation could occasionally be delayed by 6–13 days. No other side-effects were noted.

In group I (200 mg on LH + 2), treatment with mifepristone produced profound endometrial changes in all subjects with a histological pattern corresponding to the proliferative phase or irregular secretory activity. Statistical evaluation of the morphometric analysis revealed a significantly decreased glandular diameter ($P < 0.01$) and an increased number of glandular ($P < 0.01$) and stromal ($P < 0.01$) mitoses. During weekly administration of 5 mg mifepristone, statistical evaluation revealed a significant increase in number of glands ($P < 0.05$) and glandular diameter ($P < 0.02$). No statistically significant effect was observed following 2.5 mg mifepristone. Following treatment with 0.5 mg mifepristone/day, endometrial development was slightly retarded and the diameter of the glands was reduced ($P < 0.05$) from a mean of 55.5 ± 2.0 to 39.7 ± 9.7 μm after the third treatment month. No signs of hyperplasia or any endometrial atypia were found.

LIF immunostaining was observed in all controls and located to the cytoplasm of the luminal and glandular epithelial cells and stromal cells. In treatment cycles, the staining pattern of the luminal epithelium and the stroma was similar to controls (data not shown), in contrast to the glandular staining which was entirely different (Table I). Administration of 200 mg of mifepristone given on LH + 2 resulted in reduced glandular staining in all seven cases. When 5 mg of mifepristone was given once a week, all subjects showed decreased staining in the glandular epithelium in a similar way (Figure 1). In two

Table I. Immunostaining[a] of leukaemia inhibitory factor (LIF) in endometrial glandular epithelial cells in controls and during treatment with mifepristone in various doses

Subject no.	Type of cycle	Group I 200 mg (n = 7)	Group II 5 mg/week (n = 5)[b]	Group III 2.5 mg/week (n = 5)[b]	Group IV 0.5 mg/day (n = 5)[c]
1	Control	+++	+++	+++	+++
	Treatment	++	++	++	+++
	Treatment	–	++	++	++[d]
2	Control	+++	+++	+++	+++
	Treatment	++	+	+++	+++
	Treatment	–	++	+	++
3	Control	+++	++	++	++
	Treatment	++	+	+	+
	Treatment	–	++	+	*
4	Control	+++	+++	+++	+++
	Treatment	++	++	++	++
	Treatment	–	++	++	++
5	Control	++	+++	+++	+++
	Treatment	+	++	+++	++
	Treatment	–	++	++	++
6	Control	+++			
	Treatment	++			
	Treatment	–			
7	Control	+++			
	Treatment	++			
	Treatment	–			

[a]Scoring system for staining: – = absent (%), + = weak (1–30%), ++ = moderate (31–60%), +++ = strong (61–100%).
[b]Endometrial biopsies obtained during the first and second treatment cycles.
[c]Endometrial biopsies obtained during the first and third treatment cycles.
[d]Second treatment month.
*Insufficient material.

subjects (numbers 2 and 3, group II), LIF expression increased again in the second treatment month. Reduced glandular staining was also observed in all biopsies during treatment with 2.5 mg once a week and 0.5 mg of mifepristone in daily doses (Figure 2). However, in groups III and IV (2.5 and 0.5 mg) the effect seemed to be more pronounced during the second or third treatment months.

Discussion

This study was designed to evaluate the effect of the progesterone receptor inhibitor mifepristone on the expression of LIF *in vivo* in the endometrium of fertile women during the expected time of endometrial receptivity.

Evidence is accumulating to suggest that cytokines and their receptors expressed in the endometrium and embryo and controlled at the endocrine and paracrine/autocrine levels initiate the mutual recognition of implanting blastocyst and endometrium (Simon *et al.*, 1996). The coordination of cytokines and/or receptor expression by the maternal environment appears to be critical to the early development of the embryo. In mice, a burst of LIF mRNA expression restricted to the endometrial glandular epithelium occurs on day 4 of pregnancy in response to oestrogen and has been shown to be obligatory for implantation. Although the regulation of human and murine LIF is different, the role of LIF in implantation may be similar. Reverse transcriptase–polymerase chain reaction (RT–PCR) from human blastocysts shows the presence of LIF receptor mRNA only at the blastocyst stage indicating that human embryos may be capable of responding to LIF stimulus at the time of endometrial receptivity (Charnock-Jones *et al.*, 1994). The expression of LIF in human endometrium has been shown to depend on the stage of the menstrual cycle. LIF protein and mRNA concentrations increased significantly in the mid- and late secretory phase samples (Charnock-Jones *et al.*, 1994) while cultured human epithelial and stromal cells in the follicular phase secrete very low amounts of LIF (Chen *et al.*, 1995). Cultures of epithelial cells isolated from all stages of the menstrual cycle secrete more LIF than the stromal cells (Chen *et al.*, 1995).

In control biopsies taken on LH + 6 to + 8, LIF immunostaining was observed in the cytoplasm of both glands and stroma. Treatment with 200 mg of mifepristone on cycle day LH + 2 apparently had no effect on the stromal or luminal epithelial staining while reduced glandular staining was evident. The lack of effect of mifepristone on LIF expression in luminal epithelium and in stromal cells may indicate a different mechanism of regulation in these cells. It has previously been shown that steroid hormones (oestradiol and progesterone) do not have any regulatory effects on LIF mRNA expression or protein production by human endometrial cells in culture (Arici *et al.*, 1995). However, interleukin (IL-1β), tumour necrosis factor (TNF)-α, platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and transforming growth factor (TGF)-α are potent inducers of LIF expression in endometrial stromal cells. LIF expression induced by these cytokines was inhibited by interferon-γ (Arici *et al.*, 1995). Furthermore, progesterone, EGF and IL-1β up-regulate the expression of IL-1 receptor type 1 mRNA in endometrial stromal cells (Simon *et al.*, 1994a,b). The differences in the regulation of

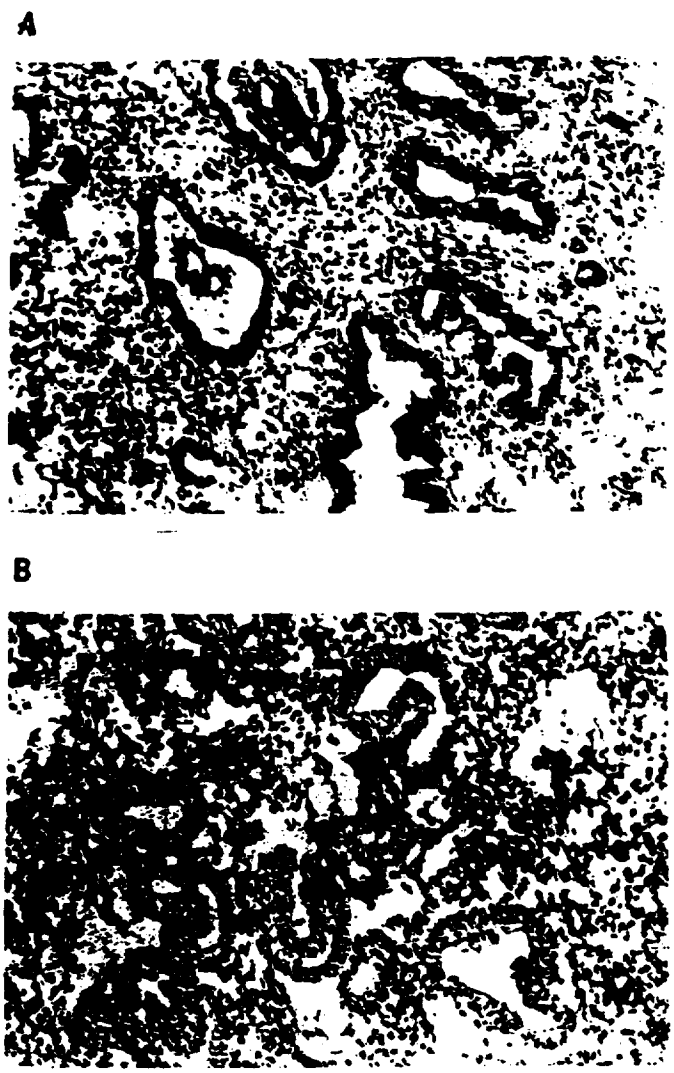

Figure 1. The effect of 5 mg mifepristone once weekly on leukaemia inhibitory factor (LIF) expression in human endometrium. An endometrial biopsy was obtained on day LH − 6 to + 8 in the control and in the second treatment cycle. (**A**) control cycle; (**B**) treatment cycle. LH = luteinizing hormone.

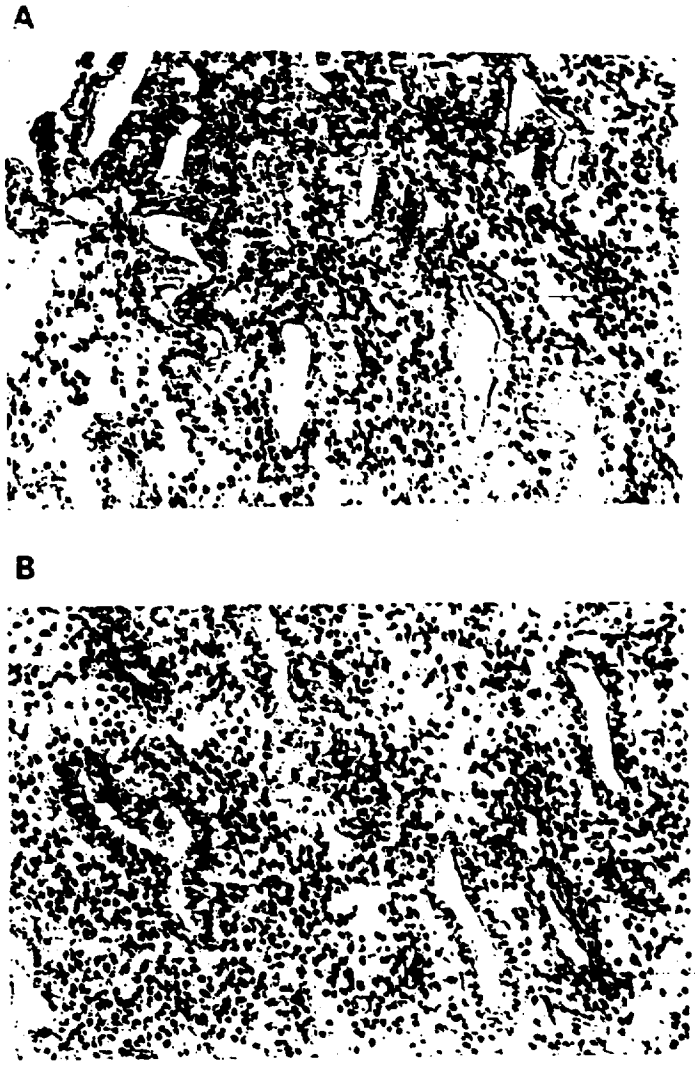

Figure 2. The effect of daily administration of 0.5 mg mifepristone on leukaemia inhibitory factor (LIF) expression in human endometrium. An endometrial biopsy was obtained on day LH − 6 to + 8 in the control and the third treatment cycle. (**A**) control cycle; (**B**) treatment cycle.

glandular stromal LIF production possibly reflect different roles for glandular and stromal cells during embryo implantation. LIF expression in glandular epithelium may initiate embryo attachment whereas, during stromal invasion, blastocyst secretion of IL-1 and other factors induce stromal secretion of LIF which then induces human chorionic gonadotrophin (HCG) production by the trophoblasts (Sawai *et al.*, 1995; Arici *et al.*, 1995).

Although glandular staining was reduced in all subjects during mifepristone treatment, staining was not completely blocked in any case. Reduced glandular staining following early luteal phase treatment with mifepristone and onapristone was recently reported by Cameron *et al.* (1996) who found that treatment with 200 mg of mifepristone or 400 mg of onapristone on cycle day LH + 2 did not significantly affect the pattern of LIF immunostaining in endometrium taken on LH + 4 while reduced staining was evident in most subjects when biopsies were taken on LH − 6. This is in accordance with our previous finding (Gemzell Danielsson *et al.*, 1993) that a single dose of 200 mg mifepristone on cycle day LH + 2 is effective for contraceptive use on a regular basis. The contraceptive effect seems to be primarily due to inhibition of endometrial maturation and function with subsequent lack of implantation.

Recent results indicate a dissociation between the central effects of mifepristone on gonadotrophin-dependent folliculogenesis and ovulation and the effect on the endometrium (Batista *et al.*, 1992; Ledger *et al.*, 1992; Croxatto *et al.*, 1993; Gemzell Danielsson *et al.*, 1996, 1997). This opens a possibility for the development of a low dose regime that would inhibit endometrial function without blocking ovulation. To investigate this possibility, mifepristone was administered once a week in 5 and 2.5 mg doses or in daily doses of 0.5 and 0.1 mg. Mifepristone in these low doses did not inhibit ovulation but, with the exception of 0.1 mg daily, significantly inhibited endometrial secretory function. Glandular expression of glycodelin and binding of *Dolichos biflorus* agglutinin (DBA) lectin was reduced as well as serum concentrations of glycodelin

Doses of 5 mg of mifepristone once a week also inhibited the normal mid-luteal down-regulation of progesterone receptors (Gemzell Danielsson *et al.*, 1996, 1997). Whether these effects on endometrial secretory activity are sufficient to prevent implantation remains to be shown. However, in the present study 5 or 2.5 mg mifepristone once a week for 2 months and 0.5 mg in daily doses for 3 months reduced glandular expression of LIF on day LH + 6 to + 8 in all subjects, apparently as effectively as treatment with 200 mg once a month. The increase in LIF staining during the second treatment month with 5 mg/week in two subjects after an initial decrease needs further elucidation. However, this could reflect the ineffectiveness of mifepristone to prevent implantation in some patients, possibly as a result of timing and dosing of drug administration.

Taken together, the results from this and previous studies indicate that endometrial LIF may also have a role in human implantation. Furthermore, this study gives additional information about the biological effect of mifepristone in human endometrium and adds further evidence to support the contraceptive potential of low dose regimes of mifepristone.

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Effect of low daily doses of mifepristone on ovarian function and endometrial development

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The effects of low daily doses of the antiprogestin mifepristone (RU 486) on ovarian and endometrial function were studied. The study included one control cycle, three treatment cycles and one follow-up cycle. During the treatment cycles, either 0.1 (n = 5) or 0.5 (n = 5) mg of mifepristone was administered once daily. Urine samples were collected three times weekly during the control and treatment cycles and pregnanediol glucuronide and oestrone glucuronide and luteinizing hormone (LH) were quantified by radioimmunoassay. Blood samples for cortisol measurement were collected once weekly and for serum glycodelin at the onset of menstruation. An endometrial biopsy was obtained in the mid-luteal phase in the control cycle and in the first and third treatment cycles and analysed by morphometric and histochemical methods. Binding of *Dolichus biflorus* agglutinin (DBA) lectin was measured and expression of progesterone and oestrogen receptors and glycodelin were analysed immunohistochemically. All cycles studied were ovulatory with an LH peak and elevated pregnanediol glucuronide concentrations. Follicular development seemed normal as judged by ultrasound examination. The length of the menstrual cycle and the menstrual bleeding were not significantly altered. Following administration of 0.5 mg mifepristone/day, endometrial development appeared to be slightly retarded and glandular diameter was significantly reduced. Furthermore, significant decreases in DBA lectin binding and endometrial expression of glycodelin were observed. Daily doses of 0.1 mg did not have any significant effect on the endometrium. No differences in oestrogen or progesterone receptor immunoactivity between control and treatment cycles were seen. This study provides further evidence that endometrial function is sensitive even to doses of antiprogestin that are low enough not to disturb ovulation. It remains to be established whether these effects are sufficient to prevent implantation.

Key words: antiprogestin/endometrial, receptivity/glycodelin/implantation/ovulation

Introduction

Mifepristone (RU 486) is a potent antigestagen that blocks progesterone action at the receptor level (Philibert *et al.*, 1982). The effects of mifepristone depend on the dose given and the stage of the menstrual cycle. Throughout the follicular phase mifepristone has little, if any, effect on the endometrium but gonadotrophin concentrations, follicular development and ovulation are suppressed (Liu *et al.*, 1987; Shoupe *et al.*, 1987b; Swahn *et al.*, 1988). Immediately after ovulation, the formation of corpus luteum is not affected, but the development of secretory endometrium is retarded (Swahn *et al.*, 1990). Later, during the luteal phase, bleeding occurs due to an effect on the endometrium, while luteal regression occurs inconsistently (Schaison *et al.*, 1985; Shoupe *et al.*, 1987a; Garzo *et al.*, 1988; Swahn *et al.*, 1988). During pregnancy, mifepristone acts on the decidua (Schindler *et al.*, 1985) and also induces myometrial activity, as well as increasing myometrial sensitivity to prostaglandins (Swahn and Bygdeman, 1988).

The various effects of antiprogestins on the hypothalamic-pituitary system and the endometrium may be useful for contraceptive purposes by inhibiting ovulation, preventing or disrupting implantation or by inducing luteal regression and menstrual bleeding. To date, mifepristone has been used as an effective postcoital method of contraception (Glasier *et al.*, 1992; Webb *et al.*, 1992) and in the early luteal phase as a once-a-month pill (Gemzell Danielsson *et al.*, 1993). If the endometrium is more sensitive to the antiprogestagenic effect of mifepristone compared to the ovary or the hypothalamic-pituitary system, it is possible that a low-dose regimen could be developed that would inhibit endometrial maturation and prevent implantation, without disturbing ovulation and the normal rhythm of the menstrual cycle. Intermittent or daily treatment would be more practical than once-a-month treatment, and would preclude failure due to possible individual variations in the receptive phase.

The aim of the present study was to evaluate this possibility. A low dose of mifepristone, 0.1 or 0.5 mg, was administered daily and the effect on ovarian function and endometrial development and function was studied.

Materials and methods

Subjects

In all, 10 healthy women were studied after giving informed consent. They were all menstruating regularly, aged 27–40 (mean 35) years, weighed 59–70 (mean 66) kg, and 0–6 (mean 3) gravida, 0–3 (mean 1.8) para. The study was approved by the ethics committee at the Karolinska Hospital, Sweden.

None of the women had used steroidal contraceptives or an intrauterine device for a minimum of 3 months prior to the study. Gynaecological examination was performed on admission. The subjects, who were not sterilized, were advised to use barrier methods for contraception during the study and all subjects were asked to keep daily records on any side-effects and bleeding. Serum chemistry analyses were done at admission and at the end of treatment.

The study included one control cycle, three treatment cycles and a follow-up cycle. During the treatment cycles, mifepristone (RU 486; Roussel Uclaf, Paris, France) was administered as daily oral doses of either 0.1 mg (n = 5) or 0.5 mg (n = 5) at 21.00 h. The follicular phase was defined as the period between the first day of menstrual bleeding (cycle day 1) and the day of urinary luteinizing hormone (LH) peak, both days inclusive. The luteal phase was defined as the period between cycle day LH+1 and the day prior to the next menstrual period, both days included. Follicular growth was monitored once weekly by pelvic ultrasonography.

Hormone determinations

Morning urine was collected three times weekly during control and treatment cycles. The samples were analysed for pregnanediol glucuronide, oestrone glucuronide and LH using radioimmunoassay (Cekan *et al.*, 1986). Hormone concentrations in the urine were expressed per gram of creatinine (Metcalf and Hunt, 1976). For creatinine analysis, a commercial kit (Sigma Diagnostics, St Louis, MO, USA, procedure no. 555) was used. In addition, all subjects determined the LH peak in urine samples collected twice daily from approximately cycle day 10 to day LH+2 by using a rapid self-test (Clearplan; Searle Unipath Ltd., Bedford, UK).

The individual steroid concentrations were normalized around the day of the LH peak, and the area under the curve was calculated by the trapezoidal method for each subject and cycle for the following periods: pregnanediol glucuronide from LH+1 to LH+11, and oestrone glucuronide and LH from LH–5 to LH+5.

Once weekly during treatment, at about 9.00 h, a blood sample was obtained for measurement of cortisol (Sufi *et al.*, 1986). Serum concentrations of glycodelin (Dell *et al.*, 1995) were measured once monthly at onset of menstruation by immunofluorometric assay (Kämäräinen *et al.*, 1994).

Endometrial biopsy

Using a Randall curette, an endometrial biopsy was obtained from the fundus and upper part of the uterus. A biopsy was taken in control, first and third treatment cycles on one of the cycle days LH+5 to LH+8 according to the LH self-test. No cervical dilatation or local anaesthesia was used. The endometrial material was assessed by morphometric and histochemical analyses.

Morphometric analyses

One part of the biopsy material was immediately fixed in Bouin's solution and used for light microscopic examination (×400) after embedding in paraffin wax and staining with haematoxylin. Morphometric analyses were performed, measuring the number of glands per mm^2, the number of glandular and stromal mitoses per 1000 glandular or stromal cells respectively, glandular diameter (mm), glandular epithelial height (mm), the number of cells with basal vacuolization per 1000 glandular cells, the number of pseudostratified cells, as well as the degree of stromal oedema, predecidualization and leukocyte infiltration. The findings were used to determine the degree of development of the endometrium and described as the day of the cycle according to Johannisson *et al.* (1987). Microscopic evaluation of the samples was performed at the end of the study by one person who was unaware of the precise cycle day and whether the biopsy had been taken in a control or a treatment cycle.

Immuno- and lectin histochemistry

A second portion of each biopsy was immediately frozen in liquid nitrogen and kept at –70°C. It was then mounted in an embedding medium which, in addition to non-reactive ingredients, contained 10.24% polyvinyl alcohol and 4.26% polyethylene glycol (O.C.T. Compound; Miles Inc., Elkhart, IN, USA) at –17°C and sectioned to 8–10 mm using a Reichert–Jung Cryocut 1800 (Cambridge Instruments GmbH, Nussloch, Germany). The sections were placed on glass slides and air dried for 15–20 min before a 10 min fixation in acetone. Thereafter, the mounted sections were wrapped in Parafilm and stored at –70°C until processed for immuno- or lectin histochemistry.

Progesterone receptors (PR) were detected using the Abbot PgR-ICA monoclonal assay system (Abbot Laboratories Inc., North Chicago, IL, USA). Localization of oestrogen receptors (ER) was performed with monoclonal antibody ER1D5 (Immunotech, SA, Marseilles, France) and the Vectastain Elite ABC immunoperoxidase detection system (Vector Laboratories Inc., Burlingham, CA, USA) according to the instructions of the manufacturer.

The secretory components of the endometrium were detected by lectin histochemistry using biotinylated *Dolichus biflorus* agglutinin (DBA) lectin at a concentration of 5 µg/ml and the Vectastain Elite ABC immunoperoxidase detection system (Vector Laboratories Inc.). DBA binds to *N*-acetylgalactosamine and galactose residues present in the glandular secretion of the mid-luteal phase endometrium (Mazur *et al.*, 1989). As a negative control, DBA was co-incubated with the corresponding carbohydrate ligand at 200 mM concentration, which completely inhibited the binding.

For the staining of glycodelin, tissue sections were treated with phosphate-buffered saline (PBS) containing Tween 20 (0.005%) for 10 min at room temperature and then incubated with normal porcine serum 1:10 in PBS for 20 min. Immunoaffinity purified polyclonal antibody against human glycodelin (Kämäräinen *et al.*, 1996) was applied (5 µg/ml) for 1 h at room temperature. After incubation, the slides were washed three times in PBS for 2 min. Specific binding of the primary antibody was detected using a complex of biotinylated porcine anti-rabbit immunoglobulins (Dako, E 353) diluted 1:300, for 40 min. The slides were washed three times with PBS for 2 min each, incubated with 0.6% hydrogen peroxide in methanol for 5 min to block endogenous peroxidase activity, and then washed again three times for 2 min each in PBS prior to incubation for 30 min with avidin–biotin complex/horseradish peroxidase (Dako Laboratories, Copenhagen, Denmark, code no. K 355) prepared according to the instructions. After three periods of 2 min each in PBS, the peroxidase substrate solution 3-amino-9-ethylcarbazole (ACE; Sigma Products, St Louis, MO, USA) was added. When incubated for 5 min the sections were rinsed with tap water, counterstained with 25% Meyers haematoxylin (Kebo, Stockholm, Sweden) for 90 s, and mounted with coverslips. Sections of human first trimester decidua were used as a positive control. As a negative control, the rabbit antiglycodelin antiserum was replaced with the immunoglobulin (Ig) G fraction of normal rabbit serum or with PBS.

Immuno- and lectin histochemical stainings were evaluated blindly by two independent persons, using a Zeiss light microscope at ×200 magnification. Observations were made in various, non-overlapping fields of the whole section in two or three labelling experiments and included stromal, luminal and glandular cells. The cells were assigned a score of 0 to 4 based on the number of cells specifically stained as follows: 0 (0% positive cells); 1, very weak (<5% positive cells); 2, weak (5–25% positive cells); 3, moderate (25–75% positive cells); or 4, strong (>75% positive cells). A similar scoring system has

been used by Press *et al.* (1988). The occurrence of specific staining for glycodelin was scored as absent (–), weak (+), moderate (++) or intense (+++).

Statistics

A log-normal distribution was assumed for the steroid hormones and LH concentrations (Gaddum, 1945). The paired *t*-test was used for evaluating differences in urinary hormone concentrations between the control and treatment cycles. Differences in morphometric parameters, receptor concentrations, DBA-lectin binding, glycodelin concentrations and the length of the menstrual cycles and bleeding were evaluated by using the two-tailed Wilcoxon's signed ranks test. A P value <0.05 was considered statistically significant. The hormonal values are presented as geometric means with 95% confidence limits.

Results

Folliculogenesis, ovulation and cycle length

All control and treatment cycles were ovulatory, with an LH peak and elevated progesterone concentrations and with follicular development judged by ultrasound. The length of the control cycle was 23–28 days. No statistical difference could be found in cycle length between control, treatment and follow-up cycles. However, one woman, while suffering from influenza, experienced a prolonged follicular phase (27 days) during the second month of daily treatment with 0.5 mg mifepristone, while the length of her luteal phase was unchanged (11 days). This woman completed only two treatment cycles and hence her second biopsy was taken during the second treatment month. The duration of menstrual bleeding (4–7 days) and subjectively evaluated blood loss were unaffected by treatment. No irregular bleeding or spotting were noted, and the results of serum chemistry analyses were within the normal range and did not differ from control values. Two patients taking the higher dose complained of acne during the first treatment month. In these subjects there were no problems during the following treatment cycles. No other side-effects were observed.

Hormone concentrations

An LH peak was detected by the self-test in all control and treatment cycles. This was also confirmed by the laboratory analyses. There was no statistical difference in the height of the LH peak (Figure 1). Urinary concentrations of oestrone glucuronide and pregnanediol glucuronide were not significantly affected by the treatment. Plasma cortisol concentrations were within the normal range and did not differ between the control and treatment cycles.

Endometrial morphometric analyses

In the control cycles, there was an excellent relationship between the calculated cycle day based on the LH peak and the histological dating of endometrial biopsies by morphometric analyses (Table I). Following treatment with 0.1 mg/day, the endometrium did not differ from the normal secretory appearance of the controls. In the higher dose group, all the parameters included in the morphometric analysis, except for glandular diameter, were unchanged. The diameter of the glands was significantly reduced ($P < 0.05$) from a mean of

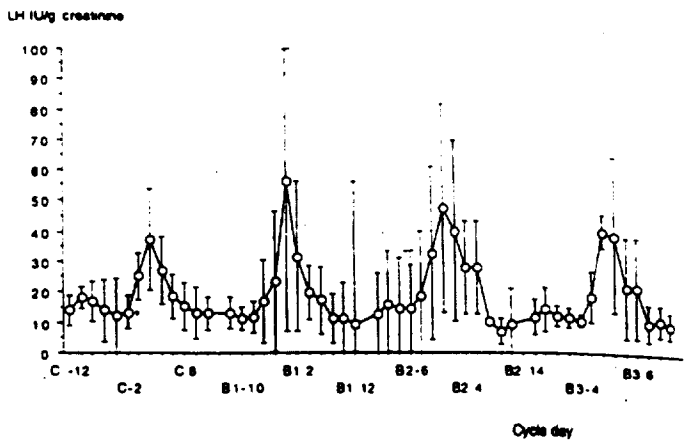

Figure 1. Urinary concentrations (geometric means and 95% confidence limits) of luteinizing hormone (LH) in control (C) and first (B1), second (B2) and third (B3) treatment cycles. Mifepristone was administered in daily doses of 0.5 mg for 3 months.

55.5 ± 12.0 to 39.7 ± 9.7 μm after the third treatment month, corresponding to a slight retardation of endometrial development.

Immuno- and lectin histochemistry

ER and PR were specifically localized in cell nuclei. In the controls, steroid receptor staining was more marked in stromal than in glandular cells. During mifepristone treatment with the higher dose (0.5 mg/day), the number of PR tended to increase in epithelial and stromal cells, but no statistically significant changes were found. The ER concentration was not affected by the treatment. In control biopsies, normal secretory function was indicated by a high degree of DBA-lectin binding. This binding was not significantly reduced after daily treatment with 0.1 mg mifepristone, while there was a significantly decreased ($P < 0.01$) binding during the third month of mifepristone treatment with 0.5 mg/day (Table II).

Glycodelin in serum

Serum concentration of glycodelin was measured at the time of menstruation (cycle day 1 ± 2 days) and was in the range of values previously reported, 22.4–74.5 μg/l (mean 45.0). (Seppälä *et al.*, 1987; Swahn *et al.*, 1993; Gemzell Danielsson *et al.*, 1996). Serum glycodelin concentrations were not affected by 0.1 mg mifepristone daily. In the higher dose group, serum glycodelin concentrations decreased slightly in four of the women (range 5.2–70.7 μg/l, mean 23.3) but increased (~2-fold) in one woman. In the latter subject, endometrial expression of glycodelin was abolished during treatment with 0.5 mg mifepristone/day.

Endometrial expression of glycodelin

In control biopsies obtained on one of the cycle days LH+5–8 (mean 7.2), all but one specimen showed positive staining for glycodelin localized within the glandular lumen. During the first treatment month with 0.1 mg mifepristone daily, no changes could be found, while the serum glycodelin concentration seemed to have decreased in the third treatment

Table I. Morphometric dating of endometrial biopsies in control and treatment cycles (0.5 mg of mifepristone daily for 3 months)

Controls			Mifepristone, 0.5 mg daily			
			1st month		3rd month	
Subject no.	Biopsy date[a]	Morphometric dating	Biopsy date[a]	Morphometric dating	Biopsy date[a]	Morphometric dating
1	+6	+6	+6	+4/5	+5	+2/3
2	+6	+6	+6	+3/4	+7	–[b]
3	+6	+6	+6	+5/6	+7	+7/8
4	+6	+6	+6	+6/7	+5	+5/6
5	+5	+4/5	+8	+6/7	+5[c]	+3/4

[a]Days after urinary luteinizing hormone peak.
[b]Insufficient material.
[c]Second biopsy taken during second month (see text).

Table II. *Dolichus biflorus* agglutinin staining intensity in endometrial glandular epithelium in control cycles and in treatment cycles in which 0.1 or 0.5 mg of mifepristone was administered once daily for 3 months ($n = 5$ in each treatment group)

Mifepristone dose (mg)	Mean (range) labelling score		
	Control cycle	1st treatment month	3rd[a] treatment month
0.1	3.4 (3–4)	3.2 (2–4)	2.9 (2–3)
0.5	3.2 (3–4)	1.1 (0–2)	0.9 (0–2)[b]

[a]One patient had the second biopsy taken during the second month (see text).
[b]$P < 0.01$ (control compared to treatment months).

Table III. Endometrial glycodelin in control and mifepristone-treated cycles as detected immunohistochemically

Subject	Presence of glycodelin[a]		
	Control	1st month	3rd[b] month
0.1 mg mifepristone			
1	++	++	++
2	+++	++	–
3	+++	+++	+
4	++	++	–
5	+	–	++
0.5 mg mifepristone			
6	++	–	–
7	+++	–	–
8	++	–	+
9	+	+	–
10	–	+	–

[a]Cells were assigned a score as follows: – = absent, + = weak, ++ = moderate, +++ = intense.
[b]One patient receiving 0.5 mg mifepristone had the second biopsy taken during the second month (see text).

cycles. The effect was more pronounced after treatment with the higher dose of 0.5 mg per day ($P < 0.05$; Table III and Figure 2). The single negative control sample was collected on day LH+6. This subject had a weak (+) positive staining for glycodelin in her first treatment cycle (0.5 mg/day) when the biopsy was obtained on cycle day LH+7. In the last treatment cycle, the endometrial tissue was collected on LH+6 and no staining was seen.

Discussion

Since progesterone is necessary for the establishment and maintenance of pregnancy, antiprogestins such as mifepristone could be expected to have a number of effects on reproductive events. Today, mifepristone in combination with a prostaglandin is a well accepted and effective non-surgical method for termination of early pregnancy (see Bygdeman, 1995). Studies on the effect of mifepristone on the menstrual cycle have shown that this agent, by blocking the PR, could interfere with both the ovarian and the endometrial function, depending on the dose and time of administration. Ovulation can be consistently suppressed by continuous administration of mifepristone in doses as low as 2 mg/day (Ledger *et al.*, 1992; Croxatto *et al.*, 1993) or 10 mg once a week (Spitz *et al.*, 1993). It has previously been shown that treatment with a high dose of mifepristone (200 g) on day LH+2 will result in lack of endometrial secretory activity, inhibited expression of 17β-hydroxysteroid dehydrogenase, inhibited down-regulation of PR (Gemzell Danielsson *et al.*, 1994; Mäentausta *et al.*, 1993), decreased concentrations of prostaglandin $F_{2\alpha}$ in uterine fluid (Gemzell Danielsson and Hamberg, 1994) and subnormal serum concentrations of glycodelin (Gemzell Danielsson *et al.*, 1996), as well as increased myometrial activity at the expected time of implantation (Gemzell *et al.*, 1990). Stimulated myometrial activity may also contribute to desynchronization between embryo and endometrium (Psychoyos and Prapas, 1987). Even much lower doses of mifepristone (down to 1.0 mg) have been shown to be sufficient to inhibit endometrial development and function, but they do not inhibit ovulation (Batista *et al.*, 1992; Croxatto *et al.*, 1993). These effects of various doses of mifepristone on its main target organs make it potentially useful for contraceptive purposes, either by its effects on folliculogenesis and ovulation (Baird *et al.*, 1995; Croxatto *et al.*, 1995; Kekkonen *et al.*, 1995) or by a direct effect on endometrium, decidua or corpus luteum to prevent implantation or interrupt early pregnancy. To date, mifepristone has only been shown to be effective for contraception when used as an emergency postcoital method (Glasier *et al.*, 1992; Webb *et al.*, 1992) or on a regular basis in the early luteal phase as a once-a-month pill (Gemzell Danielsson *et al.*, 1993).

There are now results indicating a dissociation between the central effects of mifepristone on gonadotrophin-dependent folliculogenesis–ovulation and the direct effect on the endomet-

Figure 2. Glycodelin located in glandular epithelium in control cycle (**A**) and following treatment with 0.5 mg mifepristone for 3 months (**B**). The endometrial specimens were obtained on cycle day LH+7 in both cycles. Original magnification ×125.

rium (Croxatto *et al.*, 1993; Gemzell Danielsson *et al.*, 1996). This opens the possibility for the development of a low-dose regimen that would inhibit endometrial function without blocking ovulation. In the present study, a daily dose of either 0.1 or 0.5 mg mifepristone was given for three consecutive cycles without disturbing ovulation or the normal menstrual cycle rhythm. Urinary concentrations of pregnanediol glucuronide and oestrone glucuronide after treatment remained

unchanged. Treatment with the higher dose caused a slight but obvious retardation of endometrial maturation at the expected time of implantation. This was reflected by decreased glandular diameter. In agreement with the histological changes, there was a reduced DBA binding to the endometrial glands, indicating a reduced secretory activity. Even a minor change in secretory activity may result in lack of implantation, since successful implantation depends on synchronization between the embryo and the development of a receptive endometrium (Davies *et al.*, 1990). The importance of precisely timed functioning of the endometrium is further supported by the studies of Lessey *et al.* (1995), who investigated the expression of integrins as markers of endometrial maturation and uterine receptivity.

Glycodelin (Dell *et al.*, 1995), also known as placental protein 14, is a glycoprotein with immunosuppressive and contraceptive activities (Julkunen *et al.*, 1988; Okamoto *et al.*, 1991; Oehninger *et al.*, 1995). In the endometrium, glycodelin is secreted into the glandular lumen and uterine fluid around the peri-implantation period. Glycodelin concentrations in serum start to rise in the mid-luteal phase, reaching a maximum at the onset of the next menstrual period. If the luteal phase is inadequate, the circulating concentrations are lower (Joshi *et al.*, 1986). Reduction in serum glycodelin following treatment with mifepristone has previously been reported (Gemzell Danielsson *et al.*, 1996), and following treatment with mifepristone in combination with tamoxifen (Swahn *et al.*, 1993). However, measurements of circulating concentrations of glycodelin do not seem to predict a receptive endometrium (Wood *et al.*, 1990), and recent evidence also indicates that glycodelin is not endometrium-specific. It is synthesized in haematopoetic tissues of the bone marrow (Kämäräinen *et al.*, 1994) and perhaps other tissues. A more valuable method of assessing endometrial function might be to analyse glycodelin expression in an endometrial biopsy or in uterine fluid (Rizk *et al.*, 1992; Mackenna *et al.*, 1993). Measurement of glycodelin in endometrial tissue or uterine fluid may better reflect endometrial function than the concentration in plasma, since influence by extrauterine sources of the protein is avoided.

In the present study, serum glycodelin decreased during treatment with 0.5 mg mifepristone per day, except for one subject, in whom serum concentrations increased. In this subject, endometrial glycodelin was abolished by mifepristone treatment. The increased serum concentrations may thus have been due to the increased extrauterine release of glycodelin. The endometrial expression of glycodelin was significantly decreased following daily administration of 0.5 g mifepristone. An explanation for the single glycodelin-negative control biopsy is probably that this biopsy was obtained too early (cycle day LH+6). The same woman had a weak positive biopsy during the first treatment month when the biopsy was collected on day LH+7. The results of this study lend further support to the view that progesterone plays a role in endometrial glycodelin synthesis.

In a previous study, mifepristone was given as an intermittent low dose of 2.5 or 5 mg once a week for 2 months, starting on cycle day 2 (Gemzell Danielsson *et al.*, 1996). Ovulation was not inhibited but could occasionally be delayed for 6–13 days. The length of the luteal phase was unaffected. A dose of 5 mg of mifepristone once a week was sufficient to disturb endometrial development and secretory activity significantly and to inhibit the down-regulation of PR normally occurring during the luteal phase. Endometrial morphology, PR concentration and serum concentrations of glycodelin were affected to a lesser extent with the lower dose. That the effect of low-dose treatment with mifepristone on the endometrial function observed in this and the previously mentioned study could prevent implantation is supported by the results of Katkam *et al.* (1995), who studied the effect of the antiprogestin onapristone (ZK 98.299), given in low intermittent doses to bonnet monkeys. Four animals treated with 2.5 mg onapristone for 17 cycles and another four treated with a 5 mg dose for 21 cycles did not conceive, while one animal treated with 5 mg became pregnant in the first treatment cycle. In the majority of cycles, ovulation was not disturbed but anovulation and luteal insufficiency occurred in some animals during prolonged treatment. Endometrial biopsies from 8 days after the midcycle oestradiol peak showed retardation with decreased glandular diameter.

It is unlikely that the skin problem noted by two women treated with 0.5 g/day could be a direct result of the treatment because no problems with acne were noted for daily treatment with 200 mg mifepristone in 10 patients with meningeomas (Lamberts *et al.*, 1991). Furthermore, mifepristone has very low affinity for the androgen receptor and it does not bind to transcortin or sex hormone-binding globulin (Moguilewsky and Philibert, 1985). The doses of mifepristone required to produce antiglucocorticoid effects are higher than those needed for anti-progestagenic activity (Shoupe *et al.*, 1987a). Treatment with 2–10 mg mifepristone for 30 days had no effect on the peripheral concentration of cortisol or adrenocorticotrophic hormone (Ledger *et al.*, 1992; Croxatto *et al.*, 1993), and serum cortisol was not affected by mifepristone treatment in the present study.

It is believed that progestins suppress oestrogen action in the endometrium by down-regulating ER. Daily treatment with 50 mg mifepristone for 6 months resulted in increased ER concentrations in the stroma and cystic changes consistent with oestrogenic effects (Murphy *et al.*, 1995). The significance of this 'unopposed oestrogen' effect during treatment with antiprogestin needs to be investigated further. However, both onapristone and mifepristone have the ability to antagonize the action of oestrogen on the endometrium in primates (Van Uem *et al.*, 1989). The immunoreactivity of ER and PR was unchanged after mifepristone treatment in the present study, and no signs of endometrial stimulation were found.

The present study clearly shows that endometrial secretory activity is sensitive even to such low doses of an antiprogestin that do not disturb ovarian function and ovulation. Furthermore, the concentrations of ER and PR were unaffected by the treatment and no signs of endometrial stimulation were recorded. It remains to be shown that the effects observed here on endometrial secretory activity also inhibit endometrial receptivity and implantation.

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ABSTRACT: The effect of a low dose of mifepristone (RU486) on ovarian and endometrial function was studied in 14 healthy women. The study included one control and two treatment cycles. During the treatment cycles, either 2.5 mg (n = 9) or 5 mg (n = 5) of mifepristone was administered once weekly. The concentration of ovarian steroids and luteinizing hormone (LH) in urine was measured daily, cortisol in blood once weekly and glycodelin (placental protein 14; PP14) at the time of menstruation. Ovarian function was monitored by vaginal ultrasound. An endometrial biopsy was taken in each cycle in the mid-luteal phase, based on self-measurement of the LH peak, or on cycle day 22 if no LH peak could be detected. In the evaluation of the results, the outcome of the enzyme immunoassay of LH was used to date the biopsy. Endometrial progesterone and oestrogen receptors and Dolichus biflorus agglutinin (DBA) lectin binding were measured. Ovulation was not inhibited by treatment with mifepristone, and an LH peak could be determined in all control and treatment cycles. However, in four subjects (one with the higher and three with the lower dose) the follicular phase was prolonged by 6-13 days. The duration of the luteal phase and the concentrations of pregnanediol and oestrone glucuronide were not affected by treatment. A dose of 5 mg, and to a lesser extent 2.5 mg, mifepristone once weekly caused desynchronization of endometrial development. Endometrial progesterone receptor, but not oestrogen receptor, concentration was significantly increased by the higher dose. A significant reduction in DBA-lectin binding and in serum glycodelin concentrations was also found. Thus, low doses of mifepristone do not inhibit ovulation but delay endometrial development and impair secretory activity. Whether these effects are sufficient to prevent implantation remains to be established.

Cervical Ripening With Mifepristone Before Labor Induction: A Randomized Study

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Objective: To determine the efficacy and safety of mifepristone for cervical ripening in post-term pregnancies.

Methods: Women with post-term pregnancies and Bishop scores less than 6 were assigned randomly to mifepristone (41 patients) or placebo (42 patients). Mifepristone was given orally in a dose of 400 mg. Efficacy was assessed by change in the Bishop score within 48 hours after treatment; a score of 6 or greater was considered a "strict" success. An "extended" success rate was defined, including all patients with scores of at least 6 or those who delivered within 48 hours of treatment. Antenatal safety was assessed by fetal heart rate testing before and throughout labor. Neonatal safety was assessed by Apgar score, arterial or venous pH of cord blood, and blood glucose level during the first 48 hours. Analysis used Student *t* test for continuous variables, Kruskal-Wallis test for ordinal data, and χ^2 for categoric variables.

Results: Strict success was achieved in 10 of 18 mifepristone patients (55%) evaluated for Bishop score on day 2 versus 8 of 29 placebo patients (27.5%) ($P = .004$). Extended success was achieved in 33 mifepristone patients (80.5%) and 21 placebo patients (50.0%) ($P = .004$). There were no statistical differences with regard to number of cesareans or fetal and neonatal safety.

Conclusion: Mifepristone proved effective for cervical ripening and reduced the time to delivery compared with placebo, but it did not improve the rate of cesarean. Our study did not include enough pregnancies to reach conclusions about fetal or neonatal safety. (Obstet Gynecol 1998; 92:487–92. © 1998 by The American College of Obstetricians and Gynecologists.)

Some abnormal maternal or fetal conditions require labor induction at the end of gestation, but induction can be difficult when cervical conditions are unfavorable. Prostaglandins (PGs) administered locally in the genital tract induce cervical ripening and facilitate labor induction,[1] but they sometimes cause adverse effects.

Mifepristone (RU 486) is a steroid that has antiprogesterone properties and is used for early pregnancy termination.[2] During clinical trials, it was noted that mifepristone softened and dilated the uterine cervix.[3] This effect, observed during the first and second trimesters, facilitated cervical dilation in surgical terminations of first-trimester pregnancies, reduced the doses of PG needed, and shortened the time necessary for fetal expulsion in terminations of second-trimester pregnancies.[4,5] When administered at a dose of 600 mg/day for 2 days, mifepristone is capable of inducing labor and fetal expulsion in patients with dead fetuses.[6] In a clinical study of women at term with amenorrhea ranging from 37.5 to 41.4 weeks, mifepristone was effective for initiating labor and ripening the cervix when administered in two doses of 200 mg at 24-hour intervals.[7] In light of these findings, we wanted to investigate whether a single dose of mifepristone could induce labor, ripen the uterine cervix, and facilitate induction for post-term pregnancies.

Mifepristone has antiglucocorticoid properties that show no clinical consequence in adults, even at high doses (up to 2 g in a single dose) or in prolonged administration (200 mg/day for 3 months).[8] Because it crosses the placenta,[9] its tolerance by the fetus and neonate must be evaluated carefully. The antiglucocorticoid activity of mifepristone might be responsible for hypoglycemia in neonates. The preliminary data available are reassuring; for example, in a study in monkeys,[10] the neonates were normal. In two preliminary studies in women, the neonates appeared normal and had no signs of hypoglycemia.[7,9]

The aim of this study was to evaluate the efficacy of a single dose of 400 mg of mifepristone for cervical ripening in women who required labor induction for

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post-term pregnancy and to assess its fetal and neonatal safety during the first days of life.

Subjects and Methods

From January 1991 to February 1992, this randomized, double-blind, placebo-controlled study was conducted in two centers, Montpellier University Hospital and Nantes University Hospital, France. The protocol was approved by the institutional ethics committee, and all patients gave informed written consent before inclusion. The patients were women who required labor induction for post-term pregnancies (gestational age at least 41 weeks + 3 days). Subjects eligible for inclusion were those with unfavorable cervical conditions (Bishop score less than 6) in whom labor induction could be postponed for 48 hours. Exclusion criteria included contraindication to vaginal delivery (placenta previa, breech or transverse presentation, or narrow pelvis); multiple pregnancy; uterine weakness as a result of uterine corporeal scar or high multiparity (more than four deliveries); premature rupture of the membranes; fetal heart rate (FHR) abnormality (bradycardia, variable decelerations, late decelerations, or severe tachycardia); impaired renal, adrenal, or hepatic function; corticosteroid therapy during pregnancy; and abnormality of hemostasis or anticoagulant treatment.

Forty-two subjects were included in each center, for a total of 84, divided equally between mifepristone and placebo treatments. Of these 84 women, one assigned to mifepristone could not be included because of loss of the case report form and hospital records. Therefore, analysis of efficacy and safety involved 41 women in the mifepristone group and 42 in the placebo group. Treatments were assigned randomly using a balanced randomization list obtained by permutation blocks and were administered in a one oral dose. The placebo consisted of two tablets packaged in individual bottles of identical appearance to the active product. Each bottle had a registered number in accordance with the random list. Each center was supplied with 42 packs. The randomization code for each subject was kept sealed in an opaque envelope, to be opened only in case of emergency for proper medical care of the patient. After verification of the inclusion and exclusion criteria, the patient was given an entry number and a bottle bearing the number.

Three visits were scheduled: one on day 0 for administration of 400 mg of mifepristone (Roussel-Uclaf, Paris, France) or placebo, one on day 1 for evaluation of the Bishop score (and induction if the score exceeded 6), and one on day 2 for hospitalization. In each center, Bishop scores were assigned by the same obstetricians. Each obstetrician was blinded to the group assignment, as were all persons involved in intervention (eg, nurses and pediatricians), outcome assessment, and data analysis. If labor was not induced within 48 hours after treatment, there were two possibilities. If the Bishop score was at least 6, the patient was hospitalized for labor induction with oxytocin and rupture of the membranes 2 hours after the onset of regular uterine contractions; if the Bishop score was less than 6, spontaneous induction or cervical ripening was considered to have failed. Each center was then free to use its usual induction techniques. In each center, the physician managing the patients in labor was the same one who had evaluated the Bishop scores.

Efficacy was assessed by change in the Bishop score, evaluated on day 0 before administration of treatment and on days 1 and 2. A Bishop score of 6 or more within 48 hours after administration of treatment was defined as a "strict" success of cervical ripening. An "extended" success rate was also defined, including all patients with Bishop scores of at least 6 plus any who delivered within 48 hours after treatment. Any other situation was considered a failure. Efficacy was also assessed by the interval between administration of treatment and onset of labor (effective uterine contractions with cervix dilated to 3 cm) and between administration of treatment and delivery. The time of onset of labor was assigned prospectively for each patient. The cumulative dose of oxytocin administered during labor and the number of cesareans were also used as efficacy criteria.

Antenatal safety was assessed by recording FHR on day 0 for 1 hour before treatment, on day 1, on day 2 before induction, and throughout labor. Neonatal safety was assessed by Apgar score and clinical examination at birth and by measurement of arterial or venous pH of cord blood. During the first 2 days of life, the neonatal blood glucose level was measured with Dextroxtix (Bayer SA, Diagnostics Division, Puteaux, France) once daily before one feeding bottle out of two and with the glucose oxidase method. Cortisol and mifepristone levels were measured in cord blood. A follow-up visit was scheduled for the neonate 1–2 months after birth.

Compliance with the protocol was evaluated on a case-by-case basis to assess whether observations were evaluable. The main subject characteristics in each group were compared by the Student *t* test. The Kruskal-Wallis test was used for comparison of Bishop scores on days 1 and 2, median doses of PGE_2 and oxytocin, interval between treatment and onset of labor, and interval between treatment and delivery (excluding cesarean). The distribution of intervals was compared by log-rank test. The strict and extended success rates on days 1 and 2, types of delivery, and percentages of patients receiving adjuvant treatments for induction or

Table 1. Efficacy of Mifepristone on Second Day

Characteristic	Mifepristone (n = 41)	Placebo (n = 42)	P
Total number of spontaneous labors	28 (68.3%)	14 (33.3%)	.004*
Patients with Bishop score <6 at 24 and 48 h	8 (19.5%)	21 (50.0%)	.004*
Bishop score			
Median	6	5	
Range	3–13	1–8	.035†

Data are presented as n (%) or as noted.
* χ^2 test.
† Kruskal-Wallis test.

acceleration of labor were each compared by χ^2 test. $P < .05$ was considered statistically significant.

Results

There were no statistical differences (mean ± standard deviation [SD]) between the mifepristone and placebo groups with regard to maternal age (28.5 ± 4.3 and 28.3 ± 5.0 years, respectively) or gestational age at delivery (41.5 ± 0.2 and 41.6 ± 0.2 weeks, respectively). In each group, 20 patients were nulliparas and none had previous cesareans. The distribution of pregnancies and deliveries did not differ significantly between the groups. The median Bishop score was 3 (range 1–5) in each group.

The comparative efficacy of the two regimens is reported in Table 1. Eighteen of 41 patients in the mifepristone group had an evaluation of the Bishop score on day 2, compared with 29 of 42 patients in the placebo group. Strict success for cervical ripening was achieved for ten mifepristone subjects compared with eight in the placebo group (55.5% versus 27.5%, respectively; P = .004). Spontaneous onset of labor on days 1 and 2 was significantly more frequent in patients treated with mifepristone than with placebo. Among the 23 remaining patients from the mifepristone group whose Bishop score on day 2 was not evaluated, seven delivered before day 1, 12 delivered on day 1, and four delivered on day 2 before evaluation of the score. Among the 13 patients in the placebo group whose Bishop score on day 2 was not evaluated, six delivered before day 1, four delivered on day 1, and two delivered on day 2, including one by cesarean, before evaluation of the Bishop score; one delivered after day 2 without evaluation of the Bishop score.

The extended success rate for cervical ripening within 48 hours after drug administration was also significantly higher in the mifepristone group than in the control group and was achieved for 33 patients and 21 patients, respectively (80.5% versus 50.0%; P = .004). On day 2, the need for adjuvant treatment for cervical ripening was significantly lower in the mifepristone group than the control group (19.5% versus 50%, respectively; P = .004). After the last evaluation of the Bishop score on day 2, and before cervical ripening or labor induction with oxytocin, five patients went into labor spontaneously in the mifepristone group, versus four in the placebo group. Finally, cervical ripening was obtained by intracervical administration of PGE_2 in seven patients in the mifepristone group and 17 patients in the placebo group (17% versus 40.5%, respectively; P = .004). There was no statistically significant difference between the mean dose of PGE_2 used in each group.

Onset of labor in the mifepristone group occurred significantly faster (median [range]) than in the placebo group (31.7 [9.5–117.8] versus 53.9 [2.5–192.0] hours; P = .02). The characteristics of labor are reported in Table 2. Nineteen patients in the mifepristone group and 25 in the control group received oxytocin to induce labor (46.3% versus 59.5%, respectively). Among patients who delivered vaginally, the amount of oxytocin required to induce labor and to accelerate labor was not different between the groups. Among the 33 patients with extended success in the mifepristone group, four (12.1%) cases of uterine hypertonia were reported during infusion of oxytocin. None of these women had received PGE_2. Among the 21 placebo-group patients, none experienced uterine hypertonia (P = .26). There was no statistically significant difference between the number of cesareans in the mifepristone and placebo groups (seven and six, respectively).

Two cases of FHR abnormality were noted on day 1 in the placebo group. These involved a nonreproducible deceleration observed during a contraction in one case and a moderate fetal tachycardia in the other case. No abnormalities were observed on days 0 and 2 in the two treatment groups.

During labor, 17 cases (41.5%) of FHR abnormalities were observed in mifepristone patients. These involved two cases of moderate tachycardia, five cases of nonreproducible bradycardia, three cases of nonrecurrent late decelerations, and seven cases of severe FHR abnormalities. The latter included one case of recurrent late decelerations (decelerations after three or more consecutive contractions) and six cases of progressive bradycardia. Among these seven patients, three required cesarean and four required an obstetric maneuver for delivery (forceps or vacuum extractor). Seventeen cases (40.5%) of FHR abnormalities were observed in the placebo group. These involved two cases of moderate tachycardia, three cases of nonreproducible bradycardia, five cases of nonrecurrent late decelerations, and seven cases of severe FHR abnormalities; the latter

included two cases of recurrent late decelerations and five cases of progressive bradycardia. Among these seven patients, four required cesarean and three required an obstetric maneuver for delivery (forceps, vacuum extractor, episiotomy). There was no statistically significant difference between the groups in the frequency of FHR abnormalities.

Table 3 presents neonatal outcomes. At 1 minute, three neonates (7.3%) in the mifepristone group and two (4.8%) in the placebo group had Apgar scores less than 7. At 5 minutes, all achieved Apgar scores above 7. The pH of the umbilical cord was measured in one center only and in 41 neonates. Three neonates in the mifepristone group and two in the placebo group had an umbilical artery blood pH less than 7.20, but in all cases the umbilical venous blood pH exceeded 7.20 and the Apgar score was at least 7 at 1 minute and 9 or 10 at 5 minutes. One neonate had a pH of 7.1, but the source was not specified; the Apgar scores were both 10. Considering neonatal blood glucose levels, and assuming the threshold of hypoglycemia by the glucose oxidase method as a value of 40 mg/dL, one (2.4%) and six (14.3%) cases of hypoglycemia were observed, respectively, in the mifepristone and placebo groups on the first day of life. On the second day of life, one case of hypoglycemia was observed in each treatment group.

Cortisol and mifepristone levels were measured in umbilical cord blood. Median (range) cortisol levels were higher in mifepristone patients than in placebo patients (153.5 [42–537] versus 94.5 [28–223] nmol/L). The coefficient of correlation between the time to delivery and cortisol levels was −0.41 (P = .012). The effect of mifepristone on the corticotropic axis was perceptible during the first 48 hours after administration; cortisol values then reverted to the values observed in the placebo group. In the placebo group, there was no correlation between time to delivery and cortisol levels (γ = .018, P = .279). The mean mifepristone levels in cord blood were approximately 0.25 mg/L within 48 hours after treatment. Levels decreased by half between 48 and 72 hours and then became very low (0.04 mg/L) after 72 hours. Mifepristone levels in cord blood decreased significantly as the interval between treatment and delivery increased (γ = −0.49, P = .005). The coefficient of correlation between the cortisol and mifepristone levels was significant (γ = 0.47, P = .007).

Postnatal safety was evaluated in 76 infants during visits scheduled for 1–2 months after birth. In total, seven abnormalities were reported, five of 38 (13.2%) in the mifepristone group and two of 38 (5.3%) in the placebo group, but this difference was not statistically significant (P = .42). In the mifepristone group, we noted one case of surgically corrected congenital heart disorder (interventricular septal defect), one case of insufficient weight gain, one case of fracture of the left clavicle with an extensive bony callus, one case of

Table 2. Labor Data

Characteristic	Mifepristone (n = 41)	Placebo (n = 42)	P
Interval between treatment and onset of labor (h)			
Median	31.7	53.9	.02*
Range	9.5–117.8	2.5–192.0	
Interval between treatment and delivery (h) (excluding cesareans)			
Median	31.3	58.5	.02*
Range	13.2–123.3	5.8–193.7	
Type of delivery			
Normal	25 (61%)	30 (71.4%)	
Instrumental	9 (22%)	6 (14.3%)	
Cesarean	7 (17%)	6 (14.3%)	NS†
Severe FHR abnormality	3	4	
Arrested dilation	4	0	
Arrested dilation plus FHR abnormality	0	1	
Failed trial of labor‡	0	1	
Cervical ripening in patients with Bishop score <6	7 (17.1%)	17 (40.4%)	NS†
Adjuvant treatment for labor induction	19 (46.3%)	25 (59.5%)	NS†
Cumulative oxytocin dose during labor (IU)			
Median	3	3	
Range	0.4–10	0.9–10	NS*

NS = not significant; FHR = fetal heart rate.
Data are presented as median and range or n (%).
* Kruskal-Wallis test.
† χ^2 test.
‡ Cervical dilatation did not exceed 3 cm, in the absence of FHR abnormality.

Table 3. Neonatal Data

Characteristic	Mifepristone (n = 41)	Placebo (n = 42)	P
Birth weight (g)	3418 ± 380	3502 ± 364	NS*
Apgar score <7			
1 min	3 (7.3%)	2 (4.8%)	NS†
5 min	0	0	
Transferred to a pediatric ward	5 (12.2%)	4 (9.5%)	NS†
Umbilical venous pH <7.2	1	0	NS†
Umbilical artery pH <7.2	3	2	NS*
Dextrostix‡ <40 mg/dL)			
Day 1	12 (29.3%)	15 (35.7%)	NS†
Day 2	7 (17.1%)	8 (19.0%)	NS†
Glycemia ≤40 mg/dL			
Day 1	1 (2.4%)	6 (14.3%)	NS†
Day 2	1 (2.4%)	1 (2.4%)	NS†

NS = not significant.
Data are presented as mean ± standard deviation or n (%).
* Student t test.
† χ^2 test.
‡ Bayer SA, Diagnostics Division, Puteaux, France.

buccal aphthosis, and one case of insufficient increase in the cranial perimeter between birth and the postnatal visit. In the placebo group, one case of hyperexcitability and one case of renal malformation (ureteropelvic obstruction) were reported.

Discussion

This study demonstrated significant efficacy of mifepristone for cervical ripening and induction of spontaneous labor within 48 hours after drug administration. Our findings are consistent with the results observed by Frydman et al,[7] although they used a different protocol. They administered 200 mg of mifepristone daily for 2 days instead of 400 mg in a single dose, and induction of labor was scheduled at 72 hours instead of 48 hours. In addition, the inclusion criteria in their study[7] were different from ours, as only 48% of the patients had post-term pregnancies and the others had various obstetric conditions (ie, preeclampsia, fetal growth retardation, fetal macrosomia, isoimmunization, or maternal disease). In their study,[7] 54.5% of patients in the mifepristone group went into labor within 72 hours after the first administration of treatment, and the mean interval (± SD) between the beginning of treatment and the onset of labor was 51.75 ± 26.75 hours. The results were slightly better in our study, with spontaneous onset of labor occurring in 68.3% of mifepristone patients and the median interval between administration of mifepristone and the onset of labor being 31.7 hours. These differences occurred in part because the subjects in our study were post-term and, therefore, labor induction was easier in them than in the patients of Frydman et al,[7] who were preterm or post-term. Administration of mifepristone in our study in a single dose instead of two doses might also have produced a more rapid effect. Differences in the maternal conditions necessitating labor induction might also explain the higher percentage of cesareans in the previous study[7]: 33% in each treatment group[7] versus 17% and 14% in the mifepristone and placebo groups, respectively, in our study. The percentages of women requiring cervical ripening by PGE_2 were similar in the study by Frydman et al[7] and our own: 27% and 17% in the mifepristone groups, respectively, and 58% and 40% in the placebo groups, respectively.

In the previous study[7] and ours, no differences were observed between the mifepristone and placebo groups in the percentages of FHR abnormalities or neonatal pathologic conditions. In our study, neonatal hypoglycemia was not more frequent in the mifepristone group than in the placebo group, so the antiglucocorticoid activity of mifepristone does not appear to have adverse effects on blood glucose regulation in neonates.

In our study, more women had uterine hypertonia after oxytocin in the mifepristone group than with placebo: four of 33 versus zero of 21 patients, respectively. Uterine hypertonia is a known complication of oxytocin administration. Given the limited number of patients and the lack of significant difference ($P = .26$) between the mifepristone and placebo groups, it is difficult to assess whether pretreatment with mifepristone increases the risk of uterine hypertonia during oxytocin infusion. Power analysis indicated that 42 patients would be required in each group to confirm this difference, with an α error of 5% and a β error of 10%. In the study reported by Frydman et al,[7] no case of uterine hypertonia was reported. In a study conducted in the second trimester of pregnancy,[11] pretreatment with mifepristone sensitized the uterine muscle to the action of PGs but not oxytocin. The response may be different in the third trimester of pregnancy because of the increase in the number of oxytocin receptors. Therefore, it is important to monitor uterine activity closely during oxytocin administration in patients pretreated with mifepristone.

Our study had at least one limitation. This study was done to provide preliminary results about the safety and efficacy of a single oral dose of mifepristone. The data concerning rare complications or events should be interpreted with caution. It should be noted that had more women been enrolled, a statistically significant difference between the groups might have been observed regarding criteria for antenatal and neonatal safety. Power analysis indicated that 1890 patients would be required in each group to detect a 2.5% difference in Apgar score at 1 minute, with an α error of 5% and a β error of 10%. Concerning postnatal safety, power analysis indicated that 270 patients would be required in each group to detect an 8% difference, with an α error of 5% and a β error of 10%. Further studies on larger numbers of patients should be done to determine the optimum dose of mifepristone for cervical ripening before labor induction, to assess the rate of antenatal or perinatal complications, and to compare mifepristone with other regimens that induce cervical ripening and labor.

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ABSTRACT: The author conducted a survey of pharmacists' beliefs regarding abortion and mifepristone (RU-486) to determine pharmacists' perceptions of the ethical dilemmas posed by the use of abortifacients in the United States and to determine whether and how these ethical dilemmas affect practice. The sample was nonrandom and included religiously oriented pharmacists from a variety of practice settings, approximately two-thirds of whom were women. The results indicated a plurality of beliefs about abortion rarely evident in the public debate. Although the sample supported pharmacists' right to refuse to dispense abortifacients, slightly more than half (51%) of the respondents stated that they themselves would not refuse to dispense abortifacients. Most respondents (56%) believed that abortion should remain a legal option, with slightly fewer respondents (50%) supporting the position that RU-486 should be made available in the United States. Support for abortion was proportionate to the gravity of the reason underlying the decision to abort, with the sample tending to avoid absolutistic positions. These findings tend to dispel the stereotypes and myths regarding abortion beliefs in that two-thirds of the sample were Catholic and 96% of the sample rated religion as extremely or somewhat significant in their life.

Medical Options for Early Pregnancy Termination

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Newly developed protocols using methotrexate and misoprostol are more than 90 percent effective in terminating pregnancies of less than seven weeks of gestation. Major side effects include cramping and bleeding. In a significant minority of women, the abortion is completed only after a prolonged wait. Nonetheless, abortions completed with methotrexate and misoprostol have been well tolerated and acceptable to patients. Mifepristone (formerly called RU 486) will soon be available in the United States. When used with misoprostol, mifepristone successfully terminates 94 to 99 percent of early pregnancies.

In the United States, one half of pregnancies are unplanned and one half of women with unplanned pregnancies choose to have an abortion. As a result, 1.5 million elective abortions are performed each year.[1] A family physician is often the first person with whom a woman discusses her pregnancy. The family physician plays a significant role in confirming the pregnancy and discussing her options.

In the past two decades, various combinations of medications that induce abortion have been studied. Regimens involving mifepristone (formerly called RU 486) and misoprostol (Cytotec) are currently used worldwide.[2] Mifepristone has recently been approved for this indication by the U.S. Food and Drug Administration, although it may be some time before the drug becomes available on the U.S. market.

See editorials on pages 351 and 356.

Regimens using intramuscular methotrexate and vaginal misoprostol have been used successfully in the United States[3-5] and represent a new option for pregnancy termination available to clinicians.

Most women come for pregnancy confirmation very soon after they have missed a menstrual period. In the United States, 53 percent of abortions are already performed within eight weeks of the last menstrual period, and 89 percent of abortions are currently performed during the first trimester.[1] Medical abortion is appropriate only in pregnancies within 49 days of the last menstrual period; many women request a termination during this time.

Abortifacients

The termination of unwanted pregnancies has historically been a concern of women and health care providers. In the past several centuries, many agents and modalities have been touted for their "menses-inducing" properties.[6]

In current practice, the agents used in medical abortion are methotrexate, misoprostol and mifepristone.

METHOTREXATE

Methotrexate, a dihydrofolate reductase inhibitor, is toxic to trophoblastic tissue. It is believed that damage to the trophoblast loosens its connection to the endometrium and decreases trophoblast production of human chorionic gonadotropin. Recognition of these toxic effects led to cures of choriocarcinoma using

very high doses of methotrexate.[7] In the 1980s, methotrexate was used in the treatment of ectopic pregnancy, where it was effective and well tolerated.[8]

In 1993, the first report of methotrexate used as an abortifacient in early uterine pregnancy was published.[9] As of this writing, methotrexate use for abortion in more than 1,300 patients has been reported.

MISOPROSTOL

Misoprostol is a synthetic prostaglandin E_1 analog approved in the United States for prevention of gastric ulcers. The drug is well absorbed from the gastrointestinal tract, as well as from the vaginal mucosa. Misoprostol softens the cervix and stimulates uterine contractions; for this reason, it has been used as an adjunct in medical abortion. Although it has been used as a single agent, it is more effective when used in combination with other drugs.[10]

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METHOTREXATE PLUS MISOPROSTOL

Protocols using both methotrexate and misoprostol result in completed-abortion rates of 89 to 98 percent.[3-5] Two thirds to three quarters of women abort within 24 hours of either the first or the second dose of misoprostol.

MIFEPRISTONE

Mifepristone is an antiprogestin. By antagonizing progesterone receptors in the endometrium, mifepristone causes decidual breakdown and detachment of the embryo. It also increases myometrial response to exogenous prostaglandins. As a result, myometrial contractions are enhanced in both amplitude and frequency. These actions ultimately cause sloughing of the uterine lining and expulsion of any tissue in the uterus.

Mifepristone in combination with a prostaglandin is currently approved for use in medical abortion in France, the United Kingdom, Sweden and China. Worldwide, more than 200,000 women from 20 countries have received this combination therapy for abortion. Although FDA-approved for this purpose, mifepristone is not yet available in the United States.

Advantages of Medical Abortion

Medical abortion can be offered at an earlier stage than a suction procedure. Women choosing this approach can avoid weeks of anxiety, nausea and other pregnancy symptoms while waiting for a surgical procedure. Many women report feeling more in control when they choose a medical regimen.[11]

Medical abortion is private. Only the woman and her physician need be involved in the decision. With medical abortion, neither the woman nor her physician are in a position to be subjected to the harassment that at times has occurred at locations where surgical abortion is performed.

The current political environment has created a situation in which 84 percent of American women live in counties where

there is no surgical-abortion provider.[12] Availability of medical abortion may offer more women access to a safe procedure in a familiar environment.

Disadvantages of Medical Abortion

Medical abortion takes longer to complete than a surgical procedure, which is completed within minutes. A medical abortion is an "induced miscarriage," and the course is less predictable. Bleeding and cramping may last for several hours or days. Women may miss one or two days of work or school during this time. Nausea, vomiting and/or diarrhea also occur, although these symptoms are generally mild and well tolerated. From 10 to 20 percent of women have delays of more than two weeks before the abortion is completed. Rarely, women bleed excessively and require a suction procedure.

Surgical abortions are 98 to 99 percent successful. Regimens using methotrexate with misoprostol are 90 to 95 percent successful. The combination of mifepristone and misoprostol is more efficacious, with completed abortion rates of 94 to 99 percent.[13-15] Women who choose medical treatment must be made aware that they will need a suction procedure if the products of conception are not completely expelled.

If the medications do not induce an abortion and the woman decides to continue the pregnancy, she should be aware that fetal anomalies have been associated with use of misoprostol.[15] There is also a theoretic risk of birth defects from either methotrexate or mifepristone exposure.

Counseling

Unless it is against their personal beliefs, physicians should be prepared to counsel women about the advantages and disadvantages of medical abortion (*Table 1*). Women who have experienced

TABLE 1

Comparison of Different Forms of Pregnancy Termination

Termination type	*Efficacy (%)*	*Complications*	*Cost*
Spontaneous abortion		Bleeding and cramps; incomplete expulsion with need for suction procedure	None, if complete
First-trimester surgical abortion	98 to 99	Bleeding and cramps; retained tissue; reaction to anesthesia (rare)	$200 to $700,* depending on location and type of physician
Methotrexate/misoprostol (Cytotec)	89 to 98	Bleeding and cramps; abdominal pain; nausea and vomiting; diarrhea; prolonged wait; failure of abortion	Two to three office visits; medication costs†; transvaginal sonogram
Mifepristone‡ (formerly called RU 486)/misoprostol	94 to 99	Bleeding and cramps; abdominal pain; nausea and vomiting; diarrhea; prolonged wait; failure of abortion	Two to three office visits; medication costs; transvaginal sonogram

*—*Based on average costs in the New York City area.*

†—*Four tablets of misoprostol cost about $3.00. One dose of methotrexate costs $8.00 to $10.00. Estimated cost to the pharmacist based on average wholesale prices in Red book. Montvale, N.J.: Medical Economics Data, 1997. Cost to the patient will be higher, depending on prescription filling fee.*

‡—*Not yet available in the United States.*

either medical or surgical abortions are generally satisfied with the procedure they have chosen. A recent review of 12 studies found that 64 to 95 percent of women who had undergone a medical abortion would select that method again.[11] Psychiatric morbidity after surgical abortion is low and is no greater after medical abortion.[16]

It is important to stress to women considering abortion that, when properly performed, neither surgical nor medical abortions adversely affect future fertility. A complete discussion of contraceptive options should be part of the initial counseling session.

Family physicians should consider their personal reactions to medical abortion. Physician beliefs and preferences influence the counseling they offer to patients. If the woman's personal physician chooses not to offer abortion counseling or medical therapy, patients requesting an abortion should be referred elsewhere.

A Suggested Protocol for Medical Abortion

In all medical abortions, it is essential to obtain accurate gestational dating. Although mifepristone/misoprostol abortions are approved in England for use in pregnancies up to nine weeks' (63 days) gestational age, medical abortion is most successful within 49 days of the last menstrual period. To ensure accurate dating, vaginal sonography is recommended. A baseline serum beta-human chorionic gonadotropin (βHCG) measurement should be obtained, and a bimanual pelvic examination should be performed. Other required laboratory tests include a baseline hematocrit, and determination of blood type and Rh factor. Women who are Rh-negative should receive Rh_o (D) immune globulin (Gamulin, Hyprho-D, Rhogam).

A protocol for methotrexate/misoprostol-induced abortions is outlined in *Figure 1*. Allergy to one of the medications is a contraindication. Methotrexate is administered intramuscularly in a dosage of 50 mg per m^2 of body surface.

Patients are instructed to avoid taking folate-containing vitamins for a week after the methotrexate injection.

Six to seven days after the injection, the patient inserts four tablets of misoprostol, 200 μg each (a total of 800 μg), into the vagina; the patient may do this at home. The patient should lie down for 30 minutes after inserting the tablets. If no bleeding occurs within 24 hours, the patient may insert another four misoprostol tablets, 200 μg each.

Protocols involving mifepristone are quite similar. Mifepristone is taken orally on the first day. Three to four days later, misoprostol is inserted vaginally.

Women should be seen by their physician in one to two weeks so the completion of the abortion can be assessed. This can be done either by using vaginal ultrasonography or by checking serial βHCG levels. Women who have not bled or who have evidence of continued pregnancy can be given another dose of 800 μg of misoprostol following the same protocol used the first time.

Women whose sonograms demonstrate embryonic cardiac activity two weeks after methotrexate administration should be offered a suction procedure. Women whose sonograms reveal a nonviable pregnancy should be offered the options of a suction procedure or further trials of misoprostol.

Women undergoing medical abortion may experience severe cramps and/or bleeding. Many women achieve adequate pain control with acetaminophen (Tylenol), 1,000 mg every four to six hours (maximum dosage per 24 hours: 4 g); other women require acetaminophen with codeine. Nonsteroidal anti-inflammatory drugs should be avoided because they reduce the synthesis of prostaglandins[7]

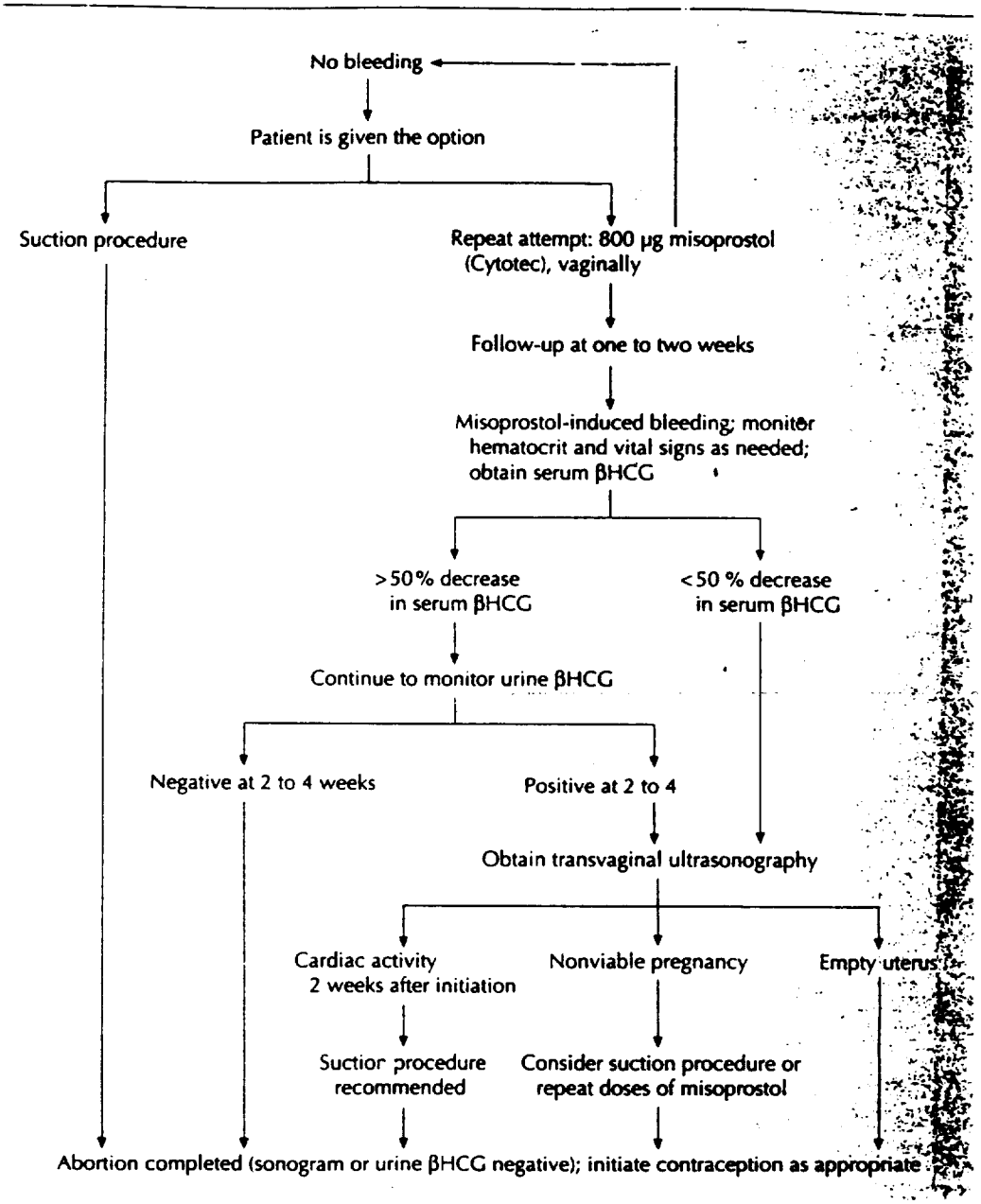

FIGURE 1. Steps to follow when initial attempt at medical abortion fails. (βHCG = beta human chorionic gonadotropin)

and may be associated with increased bleeding.

Physicians who offer medical abortion must have facilities for surgical abortion available as back-up for their patients. If they do not have the skills to perform a suction curettage, they must identify a colleague who is available to perform suction abortions.

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Pregnancy Termination

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Mifepristone (RU 486)

Current Knowledge and Future Prospects

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Mifepristone (RU 486) has received recent attention for its effects as an abortifacient. Mifepristone has not yet been approved for use in the United States. The Food and Drug Administration issued an "approvable letter" in September 1996, but mifepristone will not be available in the United States until a new manufacturer is found. Experience with mifepristone is extensive in Europe, and there have been retrospective studies and large, controlled clinical trials of its efficacy. It is most efficacious when administered to women who are less than 8 weeks pregnant, in a single 600-mg oral dose followed 48 hours later by administration of intravaginal misoprostol. This regimen has a success rate of 98%, as do most surgical abortive procedures. The most frequent adverse effect is painful contractions, which occur in up to 93% of women, with oral analgesia required in as many as half the cases. Large-scale surveys of women who elected medical abortion reported high patient satisfaction. Mifepristone is likely to have additional clinical uses. Researchers are exploring mifepristone's potential uses in cervical ripening and labor induction; contraception; delivery after intrauterine demise; treatment of breast cancer, unresectable meningioma, and prostate cancer; amelioration of endometriosis; and management of Cushing syndrome.

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Since its discovery and introduction in the early 1980s, the progesterone and glucocorticoid antagonist mifepristone (RU 486) has been studied for a myriad of clinical conditions. It has also caused great controversy. Although the drug is known (both clinically and politically) for its abortifacient effects, researchers have also studied its effects on cervical ripening, labor induction, delivery of stillborn fetuses, breast cancer, unresectable meningioma, prostate cancer, endometriosis, and Cushing syndrome.[1-17] Mifepristone has also been referred to as a contragestational agent because it prevents pregnancy both before and after conception. Until recently, mifepristone was not available for clinical use or research purposes in the United States. However, it has been prescribed for more than a decade in Europe and extensively studied in a large, unblinded, nonrandomized clinical trial in France.[1] Its pharmacology, clinical efficacy, and tolerability have been extensively characterized. Despite the highly charged political controversy, recent federal regulatory activity indicates that mifepristone will soon be available for clinical use in the United States.[2] This article will provide the practicing physician with a brief overview and summary of the clinical effects of mifepristone and what the future may hold for this compound.

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PHARMACODYNAMICS

Mifepristone was originally designed by the French pharmaceutical company Roussel-Uclaf (Romainville, France) as a glucocorticoid antagonist and was only serendipitously found to have antiprogesterone effects. Many of its poten-

rial effects are still under active research.[3] Mifepristone has 3 primary pharmacological effects: endometrial, gonadotropic, and adrenocortical.

Endometrial Effects

Mifepristone acts as a progesterone antagonist by competing with endogenous progesterone for receptor binding. It binds with very high affinity (2 to 10 times that of progesterone) to these receptors.[4] In the absence of progesterone, however, mifepristone can act as a partial agonist.[5] The putative molecular mechanism has not been proved. There is evidence that it involves a conformational change in the mifepristone-progesterone receptor complex that renders it inactive and unable to promote transcription of cellular DNA.[5] Because progesterone receptors are found primarily in reproductive organs, mifepristone exerts its principal effect on the uterus.[6] Mifepristone blocks the effects of natural progesterone on the endometrium and decidua. This leads to degeneration and shedding of the endometrial lining, thereby preventing or disrupting implantation of the conceptus.[6] Mifepristone also increases both uterine production of prostaglandins and uterine sensitivity to the contractile effects of prostaglandins.[3] It is postulated that mifepristone acts directly on the uterine muscle through an entirely separate mechanism, perhaps by increasing gap junctions in the myometrium.[6] Tissue culture studies have shown that mifepristone continues to display procontractile effects on the uterus even when the effects of prostaglandins are neutralized by treatment with indomethacin.[4]

Gonadotropic Effects

The effects of mifepristone on the hypophysial-ovarian axis have also been studied and reported in the literature. Most of these studies investigated the drug as a contraceptive as opposed to an abortifacient.[3] This distinction is important clinically as well as politically. Mifepristone has differing effects on the usual hormonal milieu when it is administered during the menstrual cycle. When given during the follicular phase it is capable of inhibiting folliculogenesis and, subsequently, the normal luteinizing hormone surge for the hypothalamus. This results in an ongoing anovular phase. Safety issues with such a major alteration in normal female hormonal patterns require further investigation.[3]

Adrenocortical Effects

Mifepristone has antiglucocorticoid effects by binding to glucocorticoid receptors with an affinity that is 2 to 3 times that of dexamethasone. It interferes with cortisol binding to tissue in the hypothalamus. This blocks normal negative feedback mechanisms and causes a compensatory increase in serum levels of both cortisol and corticotropin. In addition, the drug binds to cortisol receptors in the periphery and therefore blocks the effects of circulating cortisol in target tissue.[6] Higher doses of mifepristone are needed to produce this antiglucocorticoid effect as opposed to an antiprogestin effect.[4-6] Because blockade in the periphery is opposed by increased cortisol and corticotropin secretion, no reports of clinically significant relative cortisol deficiency have been reported when it has been used as an antiprogestin—even with long-term use of mifepristone for several weeks. Mifepristone has almost no affinity for estrogen, androgen, or mineralocorticoid receptors.

PHARMACOKINETICS

Mifepristone has a bioavailability of 70% after oral administration. Peak plasma concentrations are reached in 1 to 2 hours after a single oral dose. It has a half-life of approximately 20 to 30 hours. The pharmacokinetics of mifepristone are nonlinear. Serum drug concentrations increase progressively after oral doses from 50 to 100 mg, but no further increases occur after doses of 100 to 800 mg. This finding is partly explained by the progressive saturation of α_1-acid glycoprotein, the serum binding protein for mifepristone. The unbound mifepristone is quickly metabolized in the liver by a 2-step process, demethylation and hydroxylation, with metabolites detectable in plasma about 1 hour after oral ingestion.[7] The concentration of metabolites increases in a dose-dependent manner. Metabolites bind to progesterone receptors with an affinity of 10% to 20% that of the parent compound. These metabolites probably contribute little to the pharmacologic effect of mifepristone. Both mifepristone and its metabolites are excreted primarily in the feces via the biliary system. Little is cleared by the kidneys.[4,5,8] Mifepristone crosses the placenta. The maternal-fetal ratio in plasma is approximately 9:1.[4]

USE IN PREGNANCY TERMINATION

Most research and clinical experience with mifepristone involves its use as an abortifacient. Initial pilot studies and subsequent clinical trials have been done primarily by investigators in France. Early studies investigated the use of mifepristone (then known as RU 486) alone for the termination of early first-trimester pregnancies.[4,6,8] Success was defined as complete expulsion of the conceptus without the need for any "rescue"-type surgical procedure. All other outcomes (ongoing pregnancy, incomplete abortion, or the necessity for a hemostatic surgical procedure) were considered failures. These early studies used mifepristone in various doses over variable durations (50-800 mg over 1 to 7 days) in women with amenorrhea for less than 9 weeks. Results were variable but showed clinical success in only 50% to 85%. Success rates were lower in women with high quantitative β-human chorionic gonadotropin levels, ie, those who had been pregnant longer. In fact, those with β-human chorionic gonadotropin levels greater than 19 800 IU/L were 2.8 times as likely to have treatment fail as those with β-human chorionic gonadotropin levels less than 6358 IU/L.[8] It was subsequently shown in larger studies and by retrospective observation that the maximal success rate was achieved with a single dose of 600 mg of mifepristone administered to women who had been amenorrheic for less than 42 days (which is 2 weeks of missed menses).[4,8,9]

Prostaglandins play a major role in stimulating uterine contraction. Results of tissue studies have shown increased sensitivity of the uterus to prostaglandins when they are administered with mifepristone. Endogenous prostaglandins cause regular uterine contractions beginning from 24 to 36 hours after administration of mifepristone. These findings led to the development of sequential administration regimens of mifepristone and a low dose of prostaglandin analogue administered 36 to 72 hours later.[9] Studies using differing types of prostaglandins, administered orally, vaginally, or by the intramuscular route, showed clinical success rates of 96% or greater in women with amenorrhea for 49 days or less.[9,10,11] One study proved that vaginal administration of misoprostol (Cytotec, a synthetic prostaglandin E1 analogue; G. D. Searle & Company, Skokie, Ill) was more effective and better tolerated than oral administration.[12]

The most recently published study is a prospective clinical trial of 166 subjects in the United States. A regimen of oral mifepristone, 600 mg, followed 48 hours later by home administration of misoprostol, 800 mg (as four 200-mg tablets) intravaginally, was evaluated for pregnancy termination at up to 8 weeks of gestation (56 days or less by transvaginal sonogram).[13] Of the subjects, 82% were white, their mean age was 27 years, and the mean gestational age was 6 weeks 1 day. If the gestational sac was still present on ultrasound at 7 days, a repeated dose of misoprostol was administered. Complete abortion occurred in 98% of the subjects. Only 4% of subjects required a second dose of misoprostol.

SIDE EFFECTS AND ADVERSE OUTCOMES

The most frequent adverse effect is abdominal pain during the 4-hour period after administration of the prostaglandin analogue. This is reported in up to 93% of women and has been shown to increase proportionally with increasing prostaglandin dose.[1] Pain generally responds to oral analgesia and is significantly less than for abortive attempts using prostaglandins alone.[14]

Postcoital Contraception

Regimen	N	% of Patients		
		Failures	Nausea	Vomiting
Glasier et al[14]				
Mifepristone, 600 mg	402	0	39.5	2.6
Ethinyl estradiol, 100 µg, plus norgestrel, 1 mg	398	1.0	60.0	17.1
Webb et al[15]				
Mifepristone, 600 mg	197	3	35.5	2.5
Ethinyl estradiol, 100 µg, plus levonorgestrel, 500 µg	191	2.6	69.6	22.0
Danazol	193	4.7	30.0	3.1

In the most recent study, one third of the 166 subjects reported nausea with both medications and 68% used an oral analgesic. Other side effects include vomiting (19%), diarrhea (22%), cramping (91%), dizziness (37%), headache (19%), and fever, warmth, or chills (37%). However, 96% of the subjects agreed that the procedure "went well," and 90% agreed that home administration of misoprostol was acceptable.[13]

Serious bleeding requiring transfusion occurs in less than 1% of patients. This rate is equal to or lower than that cited for most large series of abortions by vacuum technique but underscores the need to provide ready access to after-hours care and close follow-up.[6]

It is recommended that intrauterine pregnancy be confirmed before use of mifepristone, since it is not known to be an effective treatment of ectopic pregnancy. There have been no reports of teratogenicity in humans treated with mifepristone. However, those women in whom therapy fails should undergo surgical abortion because of concerns about fetal malformations in animals.

OTHER CLINICAL USES

Postcoital Contraception/ Contragestation

Currently there are several highly effective methods in prevention of pregnancy after a single episode of unprotected intercourse. These include both high-dose estrogen alone and estrogen-progestogen combinations, sometimes referred to as "the morning-after pill." These treatments are effective only before implantation of the conceptus and are most effective within 72 hours of coitus. Mifepristone is effective regardless of implantation and can be administered up to 12 to 17 days after intercourse.[6] In repeated studies, a single 600-mg dose of mifepristone alone has been shown to be 94% to 100% effective for preventing pregnancy when administered almost anytime before expected date of menses.[3,10] Two studies compared the effectiveness of mifepristone with that of other treatments as a postcoital contraceptive (**Table**). Mifepristone was as effective as the other treatments and produced fewer side effects.[14,15]

These findings suggest that a regimen of monthly mifepristone could be used as a regular contraceptive treatment. However, monthly administration of mifepristone often alters the timing of the subsequent month's cycle, making its use difficult and impractical as a monthly birth-control device.[6,16]

Cervical Ripening

Mifepristone, as a single 600-mg dose, causes softening and dilation of the cervix. Studies have shown that the drug reduces the amount of objective and subjective force necessary to dilate the cervix in preparation for first-trimester surgical abortions without the mechanical problems of laminaria tents or the side effects and medical contraindications of prostaglandins.[5,6] Cervical softening has been shown to decrease the morbidity of the procedure. In several studies of second-trimester pregnancy termination, mifepristone administration has been shown to drastically decrease the time from prostaglandin admin-

istration to expulsion of the conceptus. At this time, however, there are only preliminary studies and more data are needed before this use can be recommended.

Intrauterine Fetal Death/Nonviable Early Pregnancy

Several studies have shown that mifepristone treatment can induce labor faster than placebo in cases of intrauterine fetal death. In addition, mifepristone may be useful in the treatment of early pregnancy failures associated with in vitro fertilization and artificial insemination or implantation techniques used for infertile couples. Nonviable pregnancies pose a risk of coagulopathy if the pregnancy remains in the uterus for more than 4 weeks. In 1 preliminary study, a single 600-mg dose of mifepristone resulted in uterine evacuation in 100% of patients with failed embryo transfer.[4-6]

Labor Induction

In a randomized, double-blind study on the proposed use of mifepristone, 200 mg daily for 2 days, for labor induction in 62 postdate pregnancies, 18 (58%) of 31 treated with mifepristone compared with 7 (23%) of 31 who received placebo went into spontaneous labor. The interval to the start of labor was shortened, the need for prostaglandin use was reduced, and the amount of oxytocin needed decreased. Questions about untoward effects on the fetus need to be resolved before this treatment can be recommended on a large scale.[4,5]

Unresectable Meningioma

Meningiomas have large concentrations of progesterone receptors. As with breast cancer, which has been shown to be responsive to antiestrogens, patients with unresectable meningiomas have been treated with mifepristone for long periods. In 1 small series of 13 patients, treatment with 200 mg of mifepristone daily resulted in minor tumor regression in 5 patients and stabilization in an additional 5.[4-6] Several other small series have also shown promise, and there is ongoing research in this area.

Endometriosis

It is known that there are progesterone receptors on endometrial implants in women with endometriosis. Small, uncontrolled trials have shown that mifepristone can decrease pain in women with diagnosed endometriosis. Yet, there was no objective decrease in the extent of ectopic endometrial implants on follow-up laparoscopy.[5]

Cushing Syndrome

The fact that mifepristone is a glucocorticoid receptor antagonist makes it a plausible drug for treating inoperable Cushing syndrome caused by ectopic corticotropin secretion or adrenocortical carcinomas. Mifepristone binds to cortisol receptors and blocks the effect of excess cortisol in the circulation. Larger doses of mifepristone must be used to obtain the antiglucocorticoid effect. Typical doses have ranged from 5 to 22 mg/kg. In a few case studies, various peripheral markers of relative hypercortisolism have improved after long-term treatment with mifepristone.[17]

CONCLUSION

No discussion of mifepristone would be complete without allusion to the intense political, ethical, and moral controversy that it has engendered. Many scientific authorities agree with Weiss[6] when he states: "If RU 486 were not an abortifacient, its other potential uses would clearly make it an important new drug, worthy of clinical investigation and possible introduction into the American pharmacopeia."[6] The discussion on either side of the issue, as with all controversy surrounding abortion, is highly charged and emotional. Mifepristone has not yet been approved for use in the United States, it will not be available in the United States until a new manufacturer is found. Mifepristone is safe for the patient and an effective abortifacient. We as clinicians must be informed about this drug—regardless of our personal views.

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Review

MEDICAL ABORTION IN EARLY PREGNANCY: A REVIEW OF THE EVIDENCE

David A. Grimes, MD

Objective: To review the literature on medical abortion in early pregnancy.

Data Sources: I performed a MEDLINE search, supplemented by bibliographies of articles and textbooks. In addition, investigators in the field were consulted to identify other sources. The review was limited to reports in English or French concerning antiprogestins or methotrexate used either alone or in combination with a prostaglandin.

Methods of Study Selection: Only those mifepristone studies with 100 or more participants were included. Those participants who received the prostaglandin sulprostone were excluded because this drug is no longer used with mifepristone. Methotrexate studies of any size were included. All reports were categorized by study type, and the evidence was evaluated using the U.S. Preventive Services Task Force rating system.

Tabulation, Integration, and Results: Both mifepristone and methotrexate, when used with a prostaglandin, can induce abortion safely in early pregnancy. Class I evidence supports a class A (good) recommendation that oral, single mifepristone doses of 200 mg and 600 mg have similar efficacy when used with a prostaglandin. Sequential and single-dose regimens have comparable efficacy. Vaginal misoprostol at 800 μg as an augmenting agent appears superior to the same dose given orally. With methotrexate abortion, 800 μg of misoprostol given vaginally 7 days after methotrexate is superior to the same dose given 3 days after. In addition, methotrexate in combination with misoprostol is more effective than misoprostol alone.

Conclusion: Medical abortion with mifepristone or methotrexate in combination with a prostaglandin is safe and effective. However, the risk of hemorrhage and gastrointestinal side effects is greater with medical abortion than with suction curettage. Further research should be done to compare mifepristone and methotrexate abortions, to determine the upper gestational age limit, and to find the best way to provide this service in the U.S. health care system. (Obstet Gynecol 1997;89:790–6. © 1997 by The American College of Obstetricians and Gynecologists.)

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In 1988, the licensing of mifepristone (RU 486) for use as a medical abortifacient in France began a new era in fertility control. Currently used for early abortion in France, the United Kingdom, Sweden, and China, mifepristone should be available soon in other European countries—and in the United States. In March 1996, the United States Food and Drug Administration received a New Drug Application from the Population Council for the marketing of mifepristone and misoprostol. The Food and Drug Administration's Reproductive Health Drug Advisory Committee has judged it safe and effective as an abortifacient, and full approval is expected. However, some U.S. and Canadian physicians, responding to patients' requests for medical abortion in early pregnancy, already have begun to offer methotrexate and misoprostol abortions as an alternative.

Women[1] and their clinicians[2] are increasingly interested in medical abortion. Depending on the gestational age limit for mifepristone abortions, as many as 800,000 U.S. women annually might be candidates for the drug; surveys suggest that use among these women may be high.[3] In anticipation of the licensing of mifepristone, this review will update clinicians about the efficacy, safety, and side effects of medical abortion with regimens using mifepristone or methotrexate. Thorough reviews of the pharmacology, mechanism of action, acceptability, and other medical uses of mifepristone have appeared elsewhere.[4–6] Although pretreatment with mifepristone can prepare the cervix for suction curettage abortion and augment labor-induction abortions, this review will consider its use only for early first-trimester abortion.

Materials and Methods

I performed a MEDLINE search back to 1980 using the key words "mifepristone," "methotrexate," and "early abortion." This was supplemented by a MEDLINE author search. Other sources included reference lists, review articles, and investigators in the field. The review was limited to reports in English and French.

I reviewed studies of the regimens of mifepristone in combination with a prostaglandin and methotrexate with or without a prostaglandin. Because sulprostone is no longer used to augment mifepristone abortions,[7] I omitted studies using this prostaglandin. Because failed attempted abortion is infrequent, I included only those mifepristone studies with 100 or more participants. However, because experience with methotrexate is lim-

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ited, I included studies of any size with this agent. I used public-domain software (EpiInfo 6; Centers for Disease Control and Prevention, Atlanta, GA) to calculate relative risks (RRs) and confidence intervals (CIs) for comparative studies, and exact binomial 95% CIs for rates of complete abortion.

Using the U.S. Preventive Services Task Force grading scheme,[8] I evaluated the quality of the evidence and the strength of recommendations possible concerning medical abortion. Class I evidence consists of at least one properly done randomized controlled trial. Class II-2 evidence includes that from well-designed cohort or case-control studies, and class III includes descriptive studies, such as case reports and case-series reports. Based on the evidence, I graded the strength of recommendation possible: A, good evidence to support a conclusion; B, fair evidence in support; and C, insufficient evidence to recommend for or against.

Results

Table 1 lists selected randomized controlled trials[9-15] that have evaluated mifepristone regimens. In 1991, the World Health Organization (WHO)[9] found similar efficacy with repeated small doses (25 mg each) of mifepristone or a single large dose (600 mg). One woman among nearly 400 required a blood transfusion.

Two trials examined the feasibility of lowering the mifepristone dose. A Scottish trial[10] showed equal efficacy with a single dose of either 200 or 600 mg. However, the modest sample size (220 participants) limited the power of the trial to detect differences. In a similar trial,[11] the WHO compared 200-, 400-, and 600-mg doses of mifepristone, each with the same gemeprost augmentation, and the three regimens had similar success rates. This latter trial had a power of 80% to detect a 5% difference in efficacy, assuming a 95% success rate with the 600-mg regimen, with α of .05. There were no important differences with respect to side effects or changes in blood pressure.

In another trial from Scotland, the prostaglandin dose was divided in an attempt to reduce the gastrointestinal side effects.[12] The efficacy of mifepristone, 200 mg orally, followed by misoprostol at 800 μg was slightly higher than that of mifepristone followed by 400 μg of misoprostol and another 400 μg in 2 hours. However, the rates of vomiting and diarrhea were higher as well.

A multicenter Chinese trial[13] examined three different regimens, each with a success rate of 94–97%. However, this study also established that misoprostol is less noxious than the prostaglandin PGO5 as an augmenting agent. For example, the RR of diarrhea among women given repeat doses of mifepristone followed by misoprostol was 0.6 (95% CI 0.4, 0.8) compared with those given the same mifepristone regimens followed by vaginal PGO5. Similarly, the RR of severe vomiting (three or more episodes) was 0.1 (95% CI 0.05, 0.4).

Investigators in Aberdeen showed the superiority of vaginal misoprostol over oral administration as an augmenting agent.[14] In this randomized controlled trial, participants received 800 μg of misoprostol, either orally or vaginally, 36–48 hours after a 600-mg oral dose of mifepristone. The vaginal route proved superior in all respects. The likelihood of needing curettage was 2.5 times higher (95% CI 1.1, 5.8) with oral than with vaginal misoprostol. Both vomiting (RR 1.4; 95% CI 1.0, 2.0) and diarrhea (RR 2.0; 95% CI 1.3, 3.1) were more frequent with oral administration of the prostaglandin.

Another randomized trial from Edinburgh[15] compared 200 mg oral mifepristone augmented 2 days later by either 0.5 mg of gemeprost vaginally or 600 μg of misoprostol orally. Rates of complete abortion up to 63 days' gestation were comparable for these regimens (97% and 95%, respectively).

Several cohort studies[16-18] have compared mifepristone regimens. Peyron et al[16] compared two different regimens of mifepristone and misoprostol. Women who received a single dose of oral misoprostol had a higher risk of failed attempted abortion (RR 2.4; 95% CI 0.9, 6.5) than those who received an optional second dose of misoprostol. With misoprostol, efficacy rates were high, yet pain appeared to be less than in studies using other prostaglandins. Gastrointestinal side effects were frequent, however.

Henshaw et al[17] provided unique information by comparing medical abortion with vacuum aspiration in the same institution. Suction curettage was more effective than mifepristone in combination with gemeprost in achieving abortion up to 63 days of amenorrhea. The likelihood of needing subsequent curettage with the medical regimen was 2.8 times that with initial suction curettage (95% CI 0.9, 8.7). In contrast, the risk of infection requiring antibiotics was lower with medical abortion (RR 0.6; 95% CI 0.3, 1.3). However, the investigators did not use prophylactic antibiotics, which reduce significantly the risk of febrile morbidity after suction curettage abortion. Significantly more women having medical abortion received parenteral analgesia (35% versus 2%).

An Indian study[18] compared four different combinations of mifepristone and vaginal meteneprost (9-methylene-PGE_2). Complete abortion rates ranged from 77% to 92%. Nine participants had to drop out of the trial because of complications: Six had profuse bleeding (one requiring transfusion) and three had excessive vomiting. One woman with a failed attempted abortion continued to term and delivered a normal infant.

Case-series reports[19-27] have examined the use of